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# PROCEEDINGS OF SYMPOSIUM ON COCCIDIODOMYCOSIS HELD AT PHOENIX, ARIZONA - FEBRUARY 11-13, 1957

Malcolm S. Ferguson

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December 1957

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# PROCEEDINGS OF SYMPOSIUM ON COCCIDIOIDOMYCOSIS

1957

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# Proceedings of Symposium On Coccidioidomycosis

**Held at Phoenix, Arizona – February 11-13, 1957**

*Sponsored by*
*The Arizona State Department of Health,*
*The Communicable Disease Center,*
*The Arizona Chapter of the American College of Chest Physicians,*
*And The Phoenix City Health Department*

**U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE**
**Public Health Service, Bureau of State Services**
**Communicable Disease Center**
**Atlanta, Georgia**

I

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PUBLIC HEALTH SERVICE PUBLICATION No. 575
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DEDICATED *to Dr. Gifford in acknowledgment of her fundamental contribution to man's understanding of coccidioidomycosis. She discovered that the benign and common San Joaquin Valley fever was actually a less virulent form of the mycotic disease that was previously thought to be rare and almost invariably fatal. This new concept led to greater recognition of the public health importance of coccidioidomycosis.*

# Foreword

Coccidioidomycosis inspired the interest of only a few investigators in the United States prior to World War II. Valley fever, desert rheumatism, or San Joaquin fever – as it was commonly known – appeared to be of local and limited concern. During the war, however, many military bases were established in the semi-arid endemic regions of the Southwest and almost overnight the disease became a major medical problem confronting the Armed Forces. Thousands of personnel stationed in the endemic areas developed disabling and occasionally fatal cases of coccidioidomycosis. Since that time, skin test surveys have shown that millions of people in this country have been infected by *Coccidioides immitis* and that thousands of new infections occur each year. In our highly mobile population, people may become infected when visiting or passing through endemic areas and move on to other parts of the country where the disease is not indigenous and hence not always suspected or recognized. The difficulty in diagnosing coccidioidomycosis and the lack of specific therapy and control measures for it present a challenge which many investigators are now attempting to meet.

In order to disseminate currently available information and to present the most recent advances resulting from research studies on coccidioidomycosis, the Arizona State Department of Health, the Arizona Chapter of the American College of Chest Physicians, and the Communicable Disease Center of the Public Health Service, in cooperation with the Phoenix City Health Department, sponsored a three-day conference on this disease. More than 250 persons attended.

Publication of these proceedings makes the wealth of material presented on the epidemiology, ecology, immunology, pathology, diagnosis, and treatment of one of man's most important mycotic diseases available to investigators everywhere.

Libero Ajello
Chairman

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## *Section I*

# Clinical, Pathological, And Laboratory Aspects Of Coccidioidomycosis

# The Public Health Significance of Coccidioidomycosis

Charles E. Smith, M.D., Demosthenes Pappagianis, Ph.D., and Margaret T. Saito, A.B.

In viewing the over-all public health significance of coccidioidomycosis, we must be careful to consider the optical system we are using. We need a microscope to detect the deaths. During World War II, despite the many hundreds of thousands of exposed susceptibles and possibly thousands of coccidioidal infections, there were only 39 deaths among our military personnel.(1) Moreover, the National Office of Vital Statistics informs us(2) that throughout the entire country in the five-year period 1951-55 there were only 353 deaths, or an average of 71 per year, attributed to coccidioidal infection. Within this period, fewer than one-half as many Americans died from coccidioidomycosis as died on the highways during the 1956 Christmas holidays.

Sixty percent of the deaths from this disease during the past five years have occurred in California.(3) Further qualitative concentration is indicated by the fact that despite far greater absolute numbers of white than nonwhite males in the endemic areas, the number of coccidioidal deaths in nonwhite males during the past five years actually exceeded those in white males by 10 percent. Moreover, though prevalence of infections is the same for both sexes and an approximately equal number of white males and white females live in endemic areas, three times as many deaths occurred in white males during the past five years as in white females. Disseminations occur especially among nonwhite males, though fortunately even among them the numbers are extremely small.

However, practically every long-time resident of Phoenix, Ariz., Bakersfield, Calif., or El Paso, Tex., will have had his personal bout with *Coccidioides*. During the dust bowl migration of the 1930's to California, approximately 8,000 new coccidioidal infections were acquired within a year and a half in two California counties. We personally saw 432 patients with allergic erythema nodosum – the "Valley Fever" manifestations – and these must have represented fully 2,000 acute respiratory coccidioidal illnesses.(4) Thus, there is a continuing burden of acute illness, although this is alleviated by our recognition that the infection is a single experience with subsequent lasting immunity. However, sooner or later one-fifth to one-quarter of the long-time residents of highly endemic areas will undergo symptomatic primary coccidioidal illnesses, well warranting the aid of physicians.(5) The infections in a few of these, notably in nonwhite males, will advance to progressive primary coccidioidal disease or coccidioidal granuloma, which is still lethal in 50 percent of the cases.

Of uncertain but more frequent occurrence and, fortunately, minimal hazard to life is the complication of coccidioidal pulmonary cavitation. In the military experience of World War [illegible], Sweigert, Turner, and Gillespie(6) reported cavitation in 8 percent of their 77 cases at Davis-Monthan Field, Arizona. There were six instances of cavitation in Willett and Weiss'(7) series of 100 patients at March Field, California. Reports by Goldstein and Louie(8) and Colburn(9) showed that of 75 cases at Camp San Luiz Obispo, California, 4 percent had cavitation. Cavities were detected in 2 percent of our series of 753 hospitalized cases at the four San Joaquin Valley Army air fields. While most of these closed during the observation period, we cannot say whether the cavities remained closed because we were unable to follow the patients after they left these fields. No data were available on cavitation occurring in other patients months later or on cavitation after inapparent infections. We can safely say that only an exceedingly small proportion of coccidioidal infections are complicated by permanent pulmonary cavities. These are not

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*These investigations were conducted under the sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, supported by the Office of the Surgeon General, Department of the Army.*

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manifestations of secondary infection, but, with possibly rare exceptions, are residual lesions of the primary infection.(10) Their deleterious effect on the health of the patient is far less than that caused by tuberculous cavities. The negligible risk of contagion likewise minimizes their public health significance. Nevertheless, by sheer force of prevalence of coccidioidal infection, cavities occur in sufficient numbers to constitute a problem and their management will be the basis of detailed discussions at this Conference. Dr. William Winn's superbly studied series again will be a central consideration. The longitudinal study of the Veterans' Administration, which Doctor Huppert will discuss, can be a mechanism for answering further questions.

Pulmonary residuals other than cavities are problems only as they constitute diagnostic enigmas. Though one or two patients apparently have experienced secondary pressure syndromes, the residuals are nearly all asymptomatic. They vex us since clinical and laboratory diagnostic procedures are seldom conclusive. Exploratory thoracotomy, if possible malignancy is a serious consideration, is the principal price which is paid and the risk is that attendant on the surgical procedure.

Public health is concerned with the mental as well as the physical health of the public. With respect to coccidioidomycosis, there are three areas of unjustified concern which constantly recur. One is an apprehension of second infections and, as a variant, whether those who have had an infection once should quit an endemic area. The second is somewhat akin, a fear of late complications, especially late disseminations. The third is the dread of contagion. Briefly, we will consider each of these.

First, is there immunity to exogenous reinfection? When we (4) analyzed our series of 432 cases of coccidioidal erythema nodosum, we noted that two patients gave histories of previous attacks of erythema nodosum which might have been diagnosed as coccidioidal "Valley Fever." In one instance the fungus was isolated during a previous attack of of erythema nodosum but was not recovered during the second and observed attack. In the other case, the fungus was demonstrated during the second and observed occurrence, but there was no recovery of the fungus and a coccidioidin test was not performed during the previous bout. In all probability, the repeated attacks merely represented different etiologies of erythema nodosum. Before we ceased handling *Coccidioides* on solid media in the laboratory, anyone joining our ranks inevitably was soon infected. However, even after sensitivity to coccidioidin waned, none of us experienced a second attack. In our own bitter experiences and those of colleagues with laboratory infections, only once have we a record of a second infection. That immunity can be broken through is shown by one instance of laboratory reinfection. A mass of spores being dried was inadvertently blown into the air. Although the person handling the spores had previously undergone an asymptomatic coccidioidal infection, he soon developed a mild illness manifested by low-grade fever and moderate malaise. He had no pleurisy, cough, or sputum. His roentgenogram remained normal but his serum developed precipitins without complement fixation and his sensitivity to coccidioidin became much greater. He also developed cross sensitivity to histoplasmin to which he had not previously reacted even when his coccidioidin test "converted" from negative to positive. Undoubtedly, without his prior asymptomatic coccidioidal infection he would have had a very severe illness, as a result of this massive exposure; as it was, there was some break-through of his immunity. However, the few spores one encounters in nature do not seem capable of producing second infections, as was indicated during the Army air field studies. The only active coccidioidal infections which were observed were in those persons untested or proved nonreactors. At times as many as one-third of the permanent personnel were coccidioidin reactors, yet none of these developed diagnosable coccidioidal reinfections. Similarly, with over 6,000 serologically diagnosed primary coccidioidal infections, we have never observed a proved second coccidioidal infection nor have we encountered a *bona fide* precipitin-positive primary infection in a person who previously had reacted to coccidioidin. Of course, it is true that precipitins may reappear during coccidioidal hydropneumothorax (10) when a cavity ruptures into the pleural space. Conceivably, a second attack of naturally acquired coccidioidomycosis could occur, or an instance of man-to-man transmission of the infection. However, such freak occurrences will not upset the general coccidioidal picture. We can reassure the patient and his family that one coccidioidal infection provides a very solid immunity. Indeed, instead of fleeing an endemic area, those once infected should be reassured about their safety from future attacks. Nature, "abhoring a vacuum," will merely replace the immune individual with a susceptible one. The excellent immunity conferred by a single attack takes a major sting from the coccidioidal public health problem. The possible disability of respiratory illness and the very slight hazard of extrapulmonary dissemination or pulmonary cavitation are limited to the single primary episode.

The second assurance we can give the person in whom the primary infection has become focalized is that his risk of late dissemination is virtually zero. At one time we feared that disseminations could occur many years after a primary infection (11) and even advised excision of a coccidioidal pulmonary cavity for this reason. (12) However, experience certainly has proved such fears to be groundless. (10,13) Exacerbation of these fears resulted from the description of lesions of varying anatomical ages in autopsy material. (14) True, we may find some lesions with fibrosis and even calcification and in the same victim of disseminating disease other lesions which are acute. However, it is unfair to draw analogies between these patients who are immunologically defective with respect to *Coccidioides* and the vast bulk of people who have effective mechanisms of immunity against it. We have records of a few patients who appear to have suffered disseminations years after their infections were acquired. Doctor Winn has one patient with a coccidioidal cavity of long duration, who later developed coccidioidal meningitis. However, some of the cases already reported are based on interpretations of data. Thus, the record of Joress,(15) whose dissemination was deemed to have occurred after several years, had already been placed in our file of disseminating infections while he still was undergoing his primary infection at Davis-Monthan Field. Another veteran was reported (16) as having developed service-acquired coccidioidal meningitis three years after his military discharge, the case being cited in support of the theory of late dissemination. However, the case history related that he had received a Certificate of Disability Discharge after his Arizona-acquired coccidioidal infection. In the subsequent three years he had complained of "nervousness and headache" with "persistent anorexia with occasional vomiting and loss of weight of 30 pounds." In a personal letter which Doctor Postlewaite had written us when he was sending us specimens from this patient he emphasized the incessant headaches for which the patient had taken "a barrel" of aspirin. No doubt he had a low-grade coccidioidal meningitis during the entire period, a theory further supported by the fact that he survived for at least another three years. As experience at the Tucson, Phoenix, and Fresno Veterans' Administration Hospitals, and even in the mildly endemic area of the San Fernando Veterans' Administration Hospital attests, veterans who undergo post-war disseminations are those who have come to endemic areas since the war. Not one of the thousands whose initial positive tests indicated they already had had primary infections when they came to the Army Air Fields experienced later disseminations. Those who disseminated had all incurred primary infections after entering military service. Indeed, the pathogenesis of the disseminations of coccidioidal granuloma closely parallels progressive primary tuberculosis both in the sequence of time and in the sites of the lesions. Very appropriate terminology would be "progressive primary coccidioidomycosis." Thus we should point out to the person undergoing his primary infection that restriction of activities as precaution against possibility of dissemination is not an excessive demand, since he is in the only vulnerable period of his infection. Subsequently, as the time interval increases, risk of dissemination will approach closer and closer to absolute zero. Hazards comparable to reinfection-type tuberculosis do not occur in coccidioidomycosis. The discussions which Doctor Robert Huntington will lead at our Conference will be most timely with respect to these aspects of pathogenesis.

The third question which plagues some people unnecessarily is the possibility of contagion. We appreciate that laboratory infections are certain if the fungus is handled on solid media, unless the greatest precautions are taken. Also, we realize that spherules are infectious, and in at least one instance their accidental introduction into the hand of a mortician (17) resulted in his primary cutaneous infection. We have yet to see a provable case of contagion.

Our studies on the epidemiology of coccidioidal erythema nodosum (4) showed that while many patients had even slept with another family member who had a bout of "Valley Fever," no infection resulted. That the family member was susceptible was established by the subsequent, unrelated attack of "Valley Fever." Rosenthal's experiments (18) in infecting guinea pigs by instilling pus down their tracheas were merely variations of Rixford's (19) original study of infection. Rosenthal's subsequently reported studies of contact between infected and uninfected guinea pigs (20) never demonstrated diagnostic endosporulating spherules or positive cultures from those animals which were deemed to have been infected by contact. We kept guinea pigs for many months in the same cages with other guinea pigs with draining testicular lesions. The normal guinea pigs did not develop skin sensitivity or positive serology, and when they were sacrificed, they had no discernible coccidioidal lesions. We attributed this absence of contagion to the comparatively large size of the spherule, which is not adapted to being air-borne. While this certainly is a logical explanation for absence of contagion during clinically manifest primary infections and during progressive primary or disseminating infections, we also know that the minute mycelial form frequently is seen in cavities. Do such arthrospores constitute hazards of contagion? Theoretically, they may. (14,21,22) Practically, they do not. (23-25) Winn, with his broad experience, has spoken decisively (13) against any evidence of contagion. Fiese,(26) in presenting his evidence against contagion, further indicates the psychological harm from useless isolation. Family contacts of patients with coccidioidal cavities who live outside the endemic area have not reacted to coccidioidin. (10-27) Within the past year we have been informed by ward physicians at Parks Air Force Base and at Oakland Naval Hospital that at each installation they had a patient with coccidioidal cavitation (one with a hydropneumothorax) with considerable production of mycelia in the sputum. However, ward attendants who had not come from known endemic areas did not react to coccidioidin. Certainly, the possibility of contagion is frequently discussed. Even outside the endemic area, the medical profession is alert to the entity of coccidioidomycosis and has diagnosed coccidioidal laboratory infections and infections acquired during single automobile or train trips through endemic areas. With such professional competence and in view of the considerable number of patients who are hospitalized or cared for in their homes outside endemic areas, contact infections would have been detected if they occurred in any significant numbers. None ever has been reported. On the other hand, we have the proved case of a man who died of disseminated disease acquired through contact with wool from the San Joaquin Valley. We know of proved primary infection acquired from exposure to the clothing and person of another individual who had dug up dirt from which *Coccidioides* was recovered. Another primary infection was traced to bolls of cotton from the endemic area. The risk from such exposures is extremely remote and no one would think of banning products from endemic areas. Neither would one consider forbidding or even discouraging travel into endemic areas – or we would not be having this Conference here. However, all these types of exposures have resulted in documented infections. By contrast, the first case of contagion has yet to be documented. May I close this phase by relating a situation which indicates that we do keep an open mind on the subject of contagion. A patient at the Fort Miley Veterans' Administration Hospital in San Francisco had genital coccidioidal dissemination with resultant *Coccidioides* in his semen. (28) We were very excited and urged that his wife be examined. However, she was of a religious group which is medically uncooperative. She would permit neither vaginal examination nor a coccidioidin test and even divorced her husband. There vanished the one case we would have felt was an outstanding possibility for contagion. May I plead that in our advice to patients and their families, as well as to less well informed professional colleagues, we reassure them that they need not be apprehensive of contagion?

What measures are applicable in meeting the problems of coccidioidal infection? Of course, the ideal would be to eradicate *Coccidioides*. However, this is unrealistic. *Coccidioides* is fully adapted to its arid habitat. A stimulating discussion of this Conference will deal with its ecology. Such studies are most appropriate. However, as we gropingly unravel this enigma, let us not delude ourselves or the public that we can accomplish more than to acquire a greater insight and respect for the organism. An organism which can grow on cacti, carrots, or ammonium acetate in water, and is flourishing over such a vast and frequently inhospitable terrain is here to stay. Philosophically, we can only be thankful that it seems more charitably inclined to man than man is to it.

If we cannot eradicate, can we alleviate the problem in nature? Yes, to a degree. Dust control will benefit those who are in endemic areas on a transient basis and the more transient the greater the benefit. Thus Emmett's studies[29] showed about one-third greater proportion of coccidioidin reactions in grade school children who were lifetime residents attending one dusty Phoenix school on the City's outskirts than in two more centrally located and less dusty Phoenix schools. Aronson's[30] earlier pioneering studies of coccidioidin sensitivity among the Arizona Indians showed that in the 2- to 4-year age group, half were coccidioidin reactors, while among those 5 to 9 years of age over nine-tenths already had acquired their infection in their very dusty environment. With most intensive dust control the annual infection rates at Minter Field in the San Joaquin Valley during World War II were reduced to approximately one-third their initial level.[31] Dust control proved to be of real value to the military, both in reducing wear and tear on engines[1] and in the reduction of disability from coccidioidomycosis. However, for lifetime residents it is immaterial whether infections occur at a rate of 25 percent per year or 8 percent per year. Ultimate infection is inevitable. Certainly, justification for lawns, paved streets, and other aspects of gracious living need not depend upon reduction of coccidioidal infection rates, though they may bring about the transfer of more clinical cases from pediatricians to internists or perhaps even to gerontologists.

Our challenge is to accept this as inevitable, to to make coexistence approach as nearly a commensal state as possible. An ideal, slim though its chances of attainment may be, is the active immunization of the susceptible population. Encouraged by the existence of coccidioidal strains known to be of low virulence,[32] we should continue our search for a completely avirulent strain which will confer a lasting immunity. Less Utopian, but nevertheless a long shot, is a non-living vaccine of sufficient durability to make its use practical. This is another subject which will be discussed in our Conference.

Our most urgent need and which, when met, will resolve our major problems will be the discovery of an antibiotic or chemotherapeutic agent fully effective against *Coccidioides immitis*. This will meet the problem of treating the tragic cases of progressive primary disseminated disease which are fortunately few. Even more importantly, the prompt treatment of early primary symptomatic infections will abort that illness and reduce the number of cases of progressive disease to minute proportions. We now possess considerable skill in diagnosing these symptomatic primary infections from which nearly all cases of progressive primary disease are subsequently derived. One hopes that the complication of coccidioidal cavitation likewise will be reduced and that treatment will be aided. The "red herrings" of coccidioidal pulmonary residuals still may annoy us, but we already appreciate that of themselves they are rarely hazards to health. Is this too optimistic? We have only to look at streptococcicosis and rheumatic fever, at tuberculosis and progressive primary tuberculosis, to see reassuring analogies. Fortunately, since the fungus does not pass from man to man, drug resistance can not be a menace as it is in staphylococcia. During the Conference vigorous discussion will focus on the searches already under way for these agents and the current studies with drugs at hand. We continue to enlist the diligence of our colleagues in pharmaceutical research, the professionals with real know-how in this basic area which is beyond the capabilities of enthusiastic but inept amateurs. We are all eager and willing to put any leads to trial. Here lies our greatest need and our major hope in meeting the public health problems of coccidioidal infections.

### Summary

Our whole country averages only 71 deaths per year from coccidioidomycosis. These deaths are concentrated in our Southwest and especially among non-white males. Virtually every person living a lifetime in endemic areas will have been infected and probably one-fifth will have symptoms of such severity as to warrant medical attention. A minute proportion then will undergo progressive primary disease with fifty percent mortality. A small proportion may be left with pulmonary residuals at the sites of the original primary pulmonary lesions. A few of these residuals may cavitate.

Fortunately, coccidioidomycosis has many alleviating aspects. Lifelong immunity is conferred by a primary infection. Furthermore, the infrequent complications are those of the primary infection. Disseminations rarely, if ever, occur years after a focalized primary infection. Moreover, even patients with *Coccidioides* in pus or sputum have never been proved to be contagious.

In assessing means of meeting the public health problem of coccidioidomycosis, we must recognize that *Coccidioides* cannot be eradicated. Dust control can reduce incidence and benefit transients, but even after extreme measures of dust control ultimately nearly all lifetime residents will undergo infection. Vaccination which could confer a durable immunity would be ideal. However, most of our problems would be solved if there were an antibiotic or chemotherapeutic agent effective in progressive primary (disseminated) disease, one safe to use during the primary infections to terminate any acute illness and prevent complications.

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# The Clinical Development and Management of Coccidioidomycosis

William A. Winn, M.D.

Inhalation of the chlamydospores or mycelial fragments of the vegetative phase of *Coccidioides immitis* by a non-immune person produces primary coccidioidomycosis. In two-thirds of the cases, the infection may manifest itself as no more than a mild or subclinical illness, rarely recognized or possibly mistaken for a light attack of the "flu." Uneventful recovery is the rule. However, it is possible that X-ray pictures of a recovered person's lungs – perhaps taken months later for some other reason – may reveal residual pulmonary changes that are the direct result of this early, undetected infection. These changes may include residual cavities, coccidioidoma, and pulmonary focal densities.

Among the remaining one-third, the illness may be sufficiently severe to cause the patient to seek medical help. The informed physician will note carefully any history of recent travel or residence in an area endemic for coccidioidomycosis, since this may provide the important clue leading to accurate diagnosis. Exposed travelers who return to their distant homes during the usual 10- to 21-day incubation period may cause the coccidioidomycosis problem to become as important to the practitioner in dear old New England and elsewhere as to those in the endemic area.

According to Birsner's(1) estimate, 25,000 to 35,000 new cases of coccidioidomycosis occur each year. A fairly significant number of these must be among vacationing travelers enjoying the attractions of the southwestern area of this country. Since their States of residence may be far from endemic areas, coccidioidomycosis assumes the stature of a problem of national importance. The establishment and continued operation of many military installations in or bordering on the endemic areas make the problem even more outstanding. Reclamation projects within the endemic desert areas involve constant tillage of the soil, and this cultivation, abetted by the recurring winds of the desert areas, provides the spore-laden dust which, as first suggested by C. E. Smith,(2) readily infects the non-immune resident or traveler. The possible existence of an animal reservoir of infection, which probably serves to continually recontaminate the soil, has been indicated in the studies of Emmons and Ashburn,(3,4) and will probably be brought out later in these discussions.

The clinical onset of coccidioidomycosis is that of a respiratory type illness, accompanied by fever and chest pain, alternating with mild chills and generalized muscular soreness, backache, and headache. Early pharyngitis may be followed by a descending bronchitis accompanied by a dry or slightly productive cough. Weakness and anorexia are usually present and may progress until the patient shows marked prostration. Arthralgia is somewhat less common and is occasionally accompanied by periarticular swelling, most frequently involving the knees and ankles. This is commonly called "desert rheumatism." Primary coccidioidomycosis is also known as "Valley Fever," "San Joaquin Fever," and somewhat inaccurately, as "California disease." The initial infection is recognized in one out of five female patients and in one out of 20 male patients because they develop erythema nodosum or erythema multiforme. Of the two skin rashes, the nodose lesions are the more common manifestation, usually appearing over the anterior surfaces of the legs and thighs, and being somewhat less frequent on the upper extremities. The lesions consist of painful, reddish-purple, nodular areas, varying in size from 1 to 10 cms. They usually persist for two or three weeks and may leave brownish-purple areas of temporary pigmentation. The multiforme type lesions, which are less frequent, are located characteristically on the upper extremities, including the face, scalp, neck, and thorax. These skin rashes mark the period of skin hypersensitivity that follows two or three weeks after the primary infection. They are important chiefly because they serve to call attention to the existence of underlying coccidioidal disease which might otherwise remain unrecognized.

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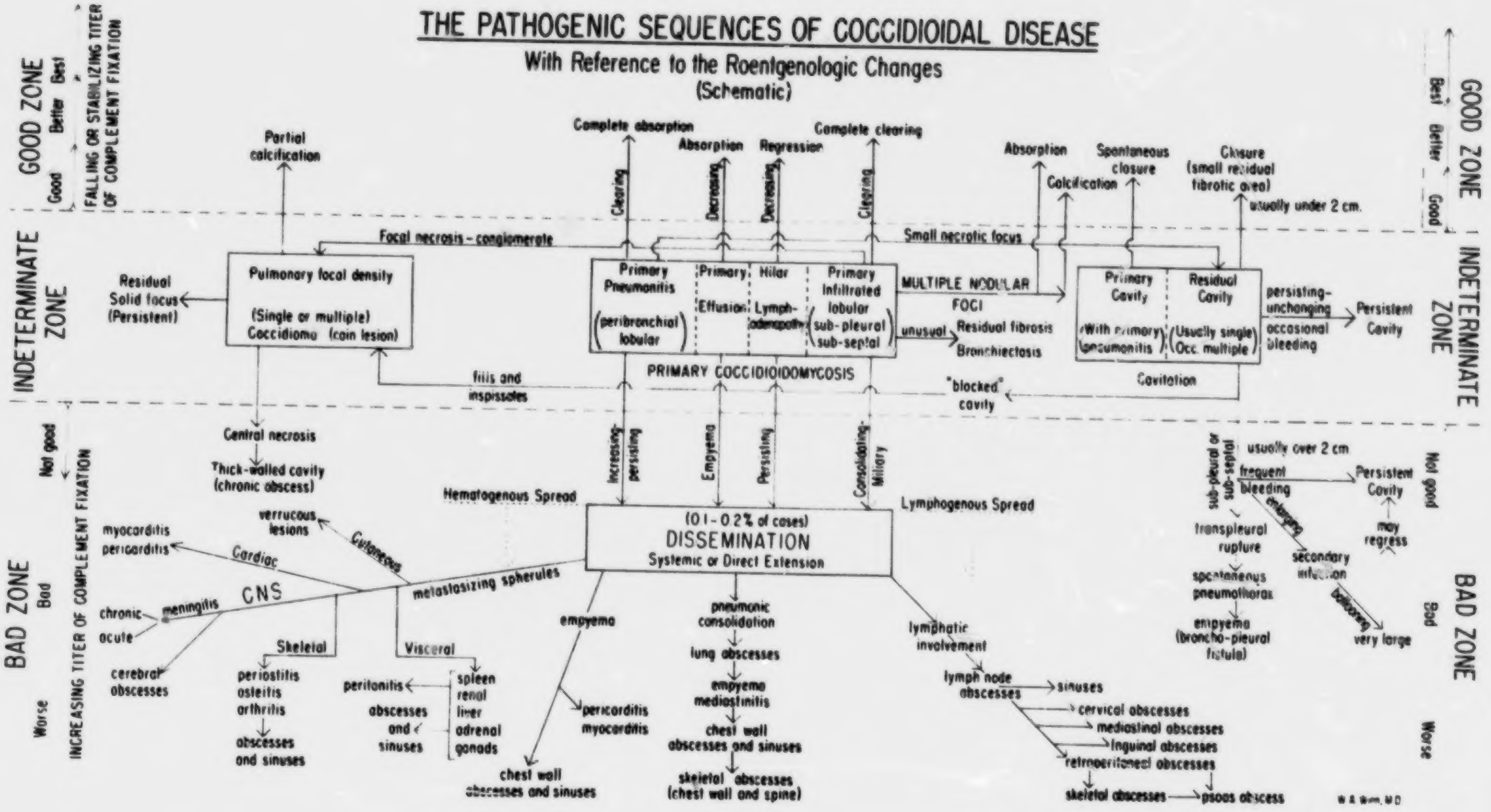
*From Tulare-Kings Counties Hospital,*
*Springville, California.*

# THE PATHOGENIC SEQUENCES OF COCCIDIOIDAL DISEASE

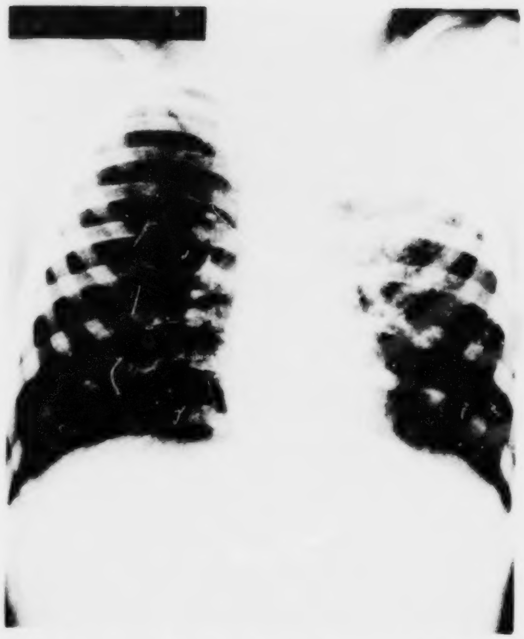
## With Reference to the Roentgenologic Changes
(Schematic)



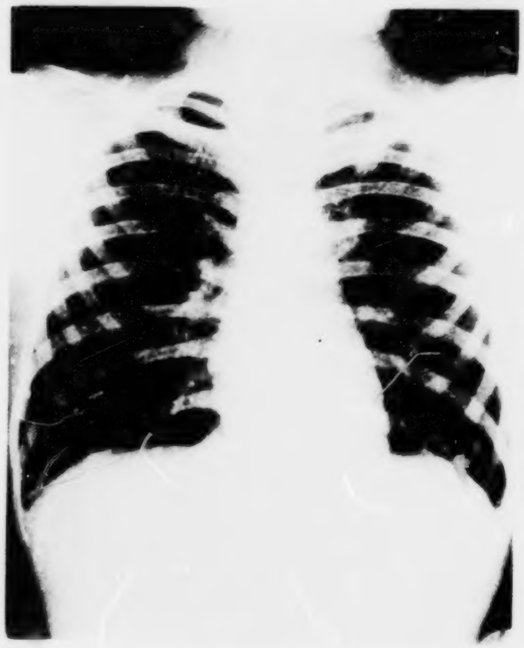
GOOD ZONE
Good
Better
Best
FALLING OR STABILIZING TITER OF COMPLEMENT FIXATION
INDETERMINATE ZONE
BAD ZONE
Not good
Bad
Worse
INCREASING TITER OF COMPLEMENT FIXATION
Partial calcification
Complete absorption
Absorption
Regression
Complete clearing
Clearing
Decreasing
Decreasing
Clearing
Focal necrosis - conglomerate
Residual Solid focus (Persistent)
Pulmonary focal density
(Single or multiple) Coccidioma (coin lesion)
Primary Pneumonitis
(peribronchial lobular)
Primary Effusion
Hilar Lymph-adenopathy
Primary Infiltrated lobular (sub-pleural sub-septal)
PRIMARY COCCIDIOIDOMYCOSIS
fills and inspissates
Small necrotic focus
MULTIPLE NODULAR FOCI
unusual
Residual fibrosis
Bronchiectosis
Absorption
Calcification
Spontaneous closure
Closure (small residual fibrotic area)
usually under 2 cm.
Primary Cavity
(With primary pneumonitis)
Residual Cavity
(Usually single) (Occ. multiple)
persisting-unchanging
occasional bleeding
Persistent Cavity
Cavitation
"blocked" cavity
Central necrosis
Thick-walled cavity (chronic abscess)
Increasing-persisting
Empyema
Persisting
Consolidating-Miliary
Hematogenous Spread
Lymphogenous Spread
(0.1 - 0.2% of cases)
DISSEMINATION
Systemic or Direct Extension
metastasizing spherules
Cutaneous
verrucous lesions
Cardiac
myocarditis pericarditis
CNS
meningitis
chronic
acute
cerebral abscesses
Skeletal
periostitis osteitis arthritis
abscesses and sinuses
Visceral
spleen renal liver adrenal gonads
peritonitis
abscesses and sinuses
empyema
pericarditis myocarditis
chest wall abscesses and sinuses
pneumonic consolidation
lung abscesses
empyema mediastinitis
chest wall abscesses and sinuses
skeletal abscesses (chest wall and spine)
lymphatic involvement
lymph node abscesses
sinuses
cervical abscesses
mediastinal abscesses
inguinal abscesses
retroperitoneal abscesses
skeletal abscesses
psoas abscess
sub-pleural or sub-septal
usually over 2 cm
frequent bleeding
Persistent Cavity
may regress
enlarging
secondary infection
ballooning
very large
transpleural rupture
spontaneous pneumothorax
empyema (broncho-pleural fistula)
W. A. Winn, M.D.

In the one-third group of clinically sick persons, a chest X-ray will usually reveal pulmonary involvement, manifested by a variety of lesions. Pneumonitis is common and when present usually produces a lobular or sublobular exudate. With more severe infections infiltrative changes, which may closely simulate tuberculosis, (5) will appear. Inflammatory reactions within the peribronchial interstitial tissues, involving the perivascular lymphatics, may result in stippling and mottling that is also very suggestive of tuberculosis. Extension to the terminal bronchioles may produce pneumonic consolidations resembling bronchopneumonia. It is believed that(6) the initial lesion is at first a low-grade bronchitis and that after enough of the spherules of *C. immitis* gain entry through the submucosa into the lymphatics of the bronchi, evidence of the disease will become visible by X-ray. Simple exudative pneumonitis usually clears in one to four weeks, but the more infiltrative type of lesions may persist longer. Consolidation of a lobe or even of an entire lung may occur and is usually followed by a severe and protracted clinical course.

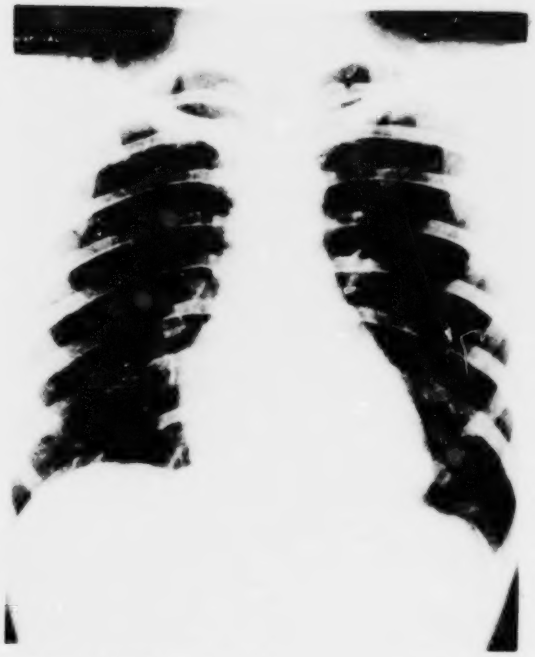
There are three types of pulmonary cavitation produced by coccidioidal disease. The first type, primary pneumonitis, may be accompanied by small, thin-walled cavities, identified as primary or acute cavities. These are transient, "will-o'-the-wisp" structures that appear and close spontaneously within a short time, and are usually not over 2 or 3 cm. in diameter. The other two types of cavities are residual lesions and will be discussed later. These include the cyst-like residual cavity and the thicker-walled, abscessing coccidioidoma. Secondary lymphadenitis, hilar and mediastinal, is a somewhat ominous roentgenographic finding which may herald dissemination. Such lung-root lymphadenitis may persist after the penumonitis has cleared, and swelling of the involved lymph nodes will persist for several months, sometimes resulting in their partial calcification. Pulmonary nodular densities (focal necrosis), single or multiple, and varying in diameter from 1.5 to 3 cm. may also accompany the primary infection. These tend to persist, absorbing very slowly, and occasionally result in permanent calcified foci. The nodules have a striking resemblance to metastatic or primary malignancy of the lung, and may cause very little symptomatology. Pleural effusion which may be the only chest pathology resulting from an initial infection is very similar to that seen in tuberculous disease. Adhesive pleuritis follows the effusion



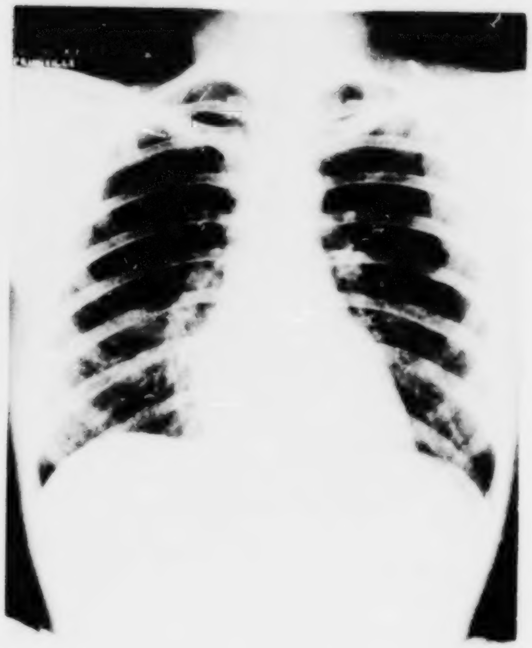
(W.T.) June 6, 1952. Primary coccidioidal pneumonitis in 12-year-old white male.



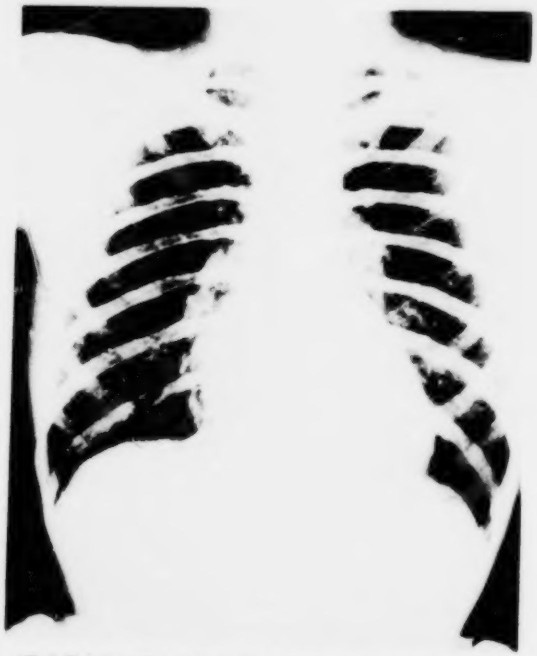
(W.T.) September 17, 1955. Same patient, 3 years and 3 months later. Clearing in left upper lobe still incomplete. (Ordinarily complete clearing occurs within 2 or 3 months.)



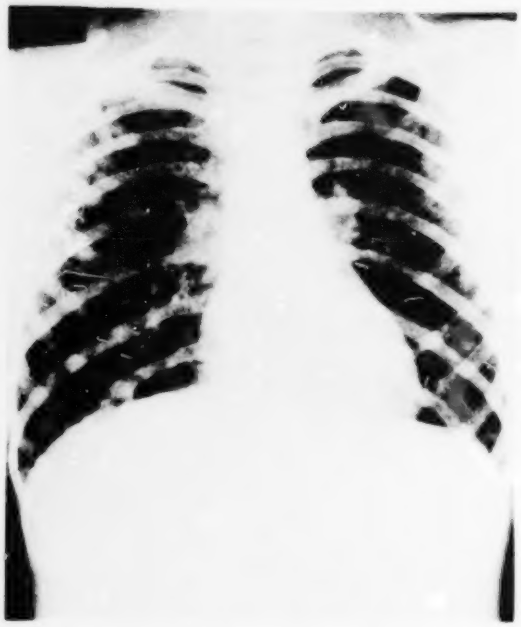
(E.J.T.) April 16, 1941. Illustrating transient cavitation occurring with primary coccidioidal pneumonitis in left apex (beneath first interspace).



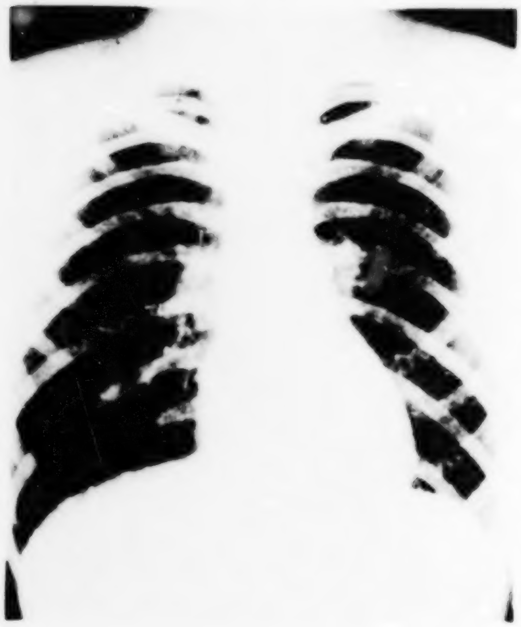
(E.J.T.) November 12, 1941. Same patient seven months later, revealing spontaneous closure of the cavity and partial absorption of density in left apex.



(E.J.T.) October 14, 1947. Same patient six and one-half years later. Small residual opacity in left first interspace. (Such "primary" cavitation usually closes spontaneously.)



(L.H.) March 23, 1954. Severe primary coccidioidomycosis with multiple nodular foci.



(L.H.) October 18, 1955. Same patient approximately 19 months later, revealing complete clearing. (In other patients such lesions have been observed to leave multiple residual calcifications.)

and may obliterate the pleural space.

These various types of pulmonary pathology(1) and their changing sequences on X-ray produce a bizarre pattern that the experienced roentgenologist will recognize as suggestive of primary coccidioidal disease.(7,8) Most of these pulmonary changes will usually clear completely, but in 5 percent of the patients there will develop static residual pulmonary lesions which persist or become visible several months later. These include both cavities and the nodular or "coin" group of lesions. The X-ray diagnostician must remain cognizant of these pulmonary sequelae to primary coccidioidomycosis.

In confirming a diagnosis of primary coccidioidomycosis one first proceeds with the coccidioidin skin test. In any respiratory-like illness, if the history suggests exposure of the patient within an endemic area, such testing should be done, regardless of whether X-ray changes are present or not. Skin-testing with coccidioidin is similar to the Mantoux procedure, except that it requires interpretation at both the 24- and 48-hour periods. One may begin with a 1:100 dilution of coccidioidin, being certain that syringes, needles, and pans have not been contaminated by any other testing agents or antigens. Skin sensitivity, manifested by induration, may not develop until the third or fourth week following infection. Therefore, if a skin test is negative early in the illness, another may be required 10 to 14 days later to help confirm a suspected diagnosis of coccidioidal disease. If the patient is found to be sensitive to coccidioidin, serological studies become important. C. E. Smith of Berkeley, Calif.(9,10) has made outstanding contributions in the development of these serologic tests, which not only confirm the diagnosis, but also indicate the severity and prognosis of the initial infection. We are fortunate that in coccidioidal disease, in contrast to other mycoses, we have such very reliable and specific serologic guidance that it is usually unnecessary to attempt to isolate the infectious agent, *C. immitis*. Cultural studies, therefore, are of limited value in the diagnosis of coccidioidomycosis. Because they can also be very hazardous to non-immune laboratory personnel, they should only be undertaken when serologic tests are negative. At least eight cases of accidental infection with one death have been due to the mishandling of cultures of *C. immitis* by laboratory personnel.(11,12)

If one must culture suspected meterial, such as bronchial washings or pus, then Sabouraud's medium may be used and incubated at room temperature until growth develops. For reasons of safety, the colony should be carefully covered with physiological saline before portions are removed for microscopic examination and animal inoculation. This is done by gently injecting the sterile saline through the cotton plug with a long hypodermic needle and syringe.

The mycelium is examined in a drop of lactophenol cotton blue mounting fluid for the presence of the diagnostic barrel-shaped arthrospores.

Pathogenicity tests should be carried out on all suspected isolates of *C. immitis*. These are done by injecting a saline suspension of mycelium and arthrospores intraperitoneally into mice or intratesticularly into guinea pigs. The discovery of endosporulating sporangia at necrospy in the animal tissues confirms or establishes identification.

The differential diagnosis of primary coccidioidomycosis includes:

1. Any of the pneumonitis-producing diseases of either bacterial or viral origin
2. Lobar or bronchopneumonia
3. Pulmonary tuberculosis
4. Other mycotic diseases including blastomycosis and histoplasmosis
5. Pleuritic effusions of tuberculous, viral, or bacterial origin
6. Pulmonary malignancies of primary and metastatic type.

What happens to the patient diagnosed as having primary coccidioidomycosis will depend upon the following factors:

1. The severity of the initial infection
2. The resistance of the host
3. Racial susceptibility
4. The adequacy of treatment, which implies early diagnosis.

The first factor, severity of the infection, will be quantitatively related to the actual number of chlamydospores that gain entry into the respiratory system, penetrate the initial defense mechanism, and successfully adapt themselves to the tissue environment by changing to their parasitic phase of development to form endosporulating spherules (sporangia). Endosporulation within the spherules causes them to enlarge until the walls burst and liberate the contained endospores. Each of these endospores repeats this spherule-endospore cycle and a rapidly self-multiplying, foreign-body type of infection results. The cellular reaction provoked thereby consists of a lymphocytic and monocytic response, with associated tubercle formation containing foreign-body or Langhans type giant cells. Usually, the tissue reaction successfully walls off the infectious process, but occasionally it fails and a break-through results. When this occurs, local extension of the infection is followed by widespread dissemination of spherules and endospores, through the lymphatic and vascular channels to distant body systems and areas.

The second factor, resistance of the host, is difficult to evaluate. It is believed that some people who live within the endemic area for many years fail to react to the coccidioidin skin test, although they must have been repeatedly exposed to air-borne chlamydospores. We know that resistance may vary according to the physiologic state of the body. An example of this is the susceptibility of the gravid female patient infected by the fungus during the first trimester of pregnancy. In some patients, delayed antibody formation with a tardy appearance of precipitins and complement fixation seems to be associated with a slow recovery from the initial infection. In others who have recovered from a severe primary infection, such as one involving the entire lung, a high titer of complement fixation may persist over a period of years, which by its very stabilization seems to insure a good prognosis. The incomplete fixation of complement, occasionally observed by C. E. Smith, is indicative of overwhelming infection or dissemination, and apparently also of an antibody type of immuno-defense mechanism that is only partly effective. In a few cases of incomplete fixation, precipitins have reappeared, accompanied by clinically progressive disease. That the titer of complement fixation is somewhat related quantitatively to the amount of diseased tissue is shown when a definite drop of the titer occurs following lobectomy.

The third factor, racial susceptibility, is generally accepted. The Filipino and Negro are known to be especially vulnerable to dissemination of the primary infection. In such dark-skinned races, dissemination occurs 20 to 30 times as often as among the light-skinned.

The fourth factor, adequacy of treatment, depends on early recognition and diagnosis, and prompt enforcement of bedrest to facilitate focalization of the initial infection. It is of paramount importance that such treatment be continued until focalization is well established. In achieving this, the clinician will be guided by a decrease in such symptoms and signs as cough, fever, chest pain, and muscular soreness. By this time, any skin rash will have faded. Serial X-ray studies will reveal clearing of the pulmonary changes, including recession of any hilar or mediastinal adenopathy. Bedrest should continue until there is an actual return of strength and a feeling of well-being, accompanied by a decreasing red-blood-cell sedimentation rate. A careful search should be made to rule out the existence of distant lesions especially within the lymphoid, skin, cardiac, skeletal, and central nervous systems. Finally, and most important, the serologic findings must indicate a successfully focalizing infection by a receding titer of complement fixation, or stabilization of the fixation in the low dilutions of serum. After clinical recovery, there must be adequate followup, including serial chest X-ray studies at quarterly intervals, to detect the appearance of late residual-type pulmonary lesions such as cavitation or coccidioidoma.

In summary, the medical management of primary coccidioidomycosis is based upon bedrest in an attempt to gain successful focalization of the initial infection. If the patient is dark-skinned, such bedrest must be strict and prolonged. If the patient is pregnant, complete bedrest again becomes mandatory.(13, 15) In order to assure adequate physical rest, analgesics, including codeine, may be needed for relief of pain. Sedation of the apprehensive patient becomes important, and careful nursing supervision is always necessary. We should insure adequate nutrition by giving a high protein, high vitamin diet, supplementing the vitamin intake with 500 mg. of vitamin C daily. Painful nodose lesions may be relieved somewhat by antihistamines. Under no circumstances should adrenal steroids or cortisone preparations be used, as these may cause rapid dissemination of a focalizing primary infection. The physician must learn to depend upon the serological studies and their prognostic value. The patient must be inspected daily for the appearance of distant lesions produced by the metastasis of spherules to other areas, particularly the lymphatic and skin systems. Metastatic skin foci will require immediate cauterization, not only to destroy these lesions, but to seal off the further spread of spherules from them via lymphatic and vascular channels. Joint or bone pains may require careful radiologic examination and splinting. Headache may require examination of the cerebrospinal fluid. In the very prostrate patient, transfusions may be helpful, particularly immunotransfusions using blood freshly obtained from a recovered person who has a stabilized high titer of complement fixation.

When focalization fails and the catastrophe of dissemination occurs, one is usually forewarned by a continued rise in the titer of complement fixation, and also by the loss of skin sensitivity to coccidioidin (anergy). The careful physician fears dissemination if prostration of the patient continues, or if a septic type of temperature reaction persists, along with an increasing blood sedimentation rate. The earliest possible interpretation of such clinical criteria enables the physician to intensify the necessary therapy. If the patient is dark-skinned, the physician will anticipate a greater tendency toward dissemination.

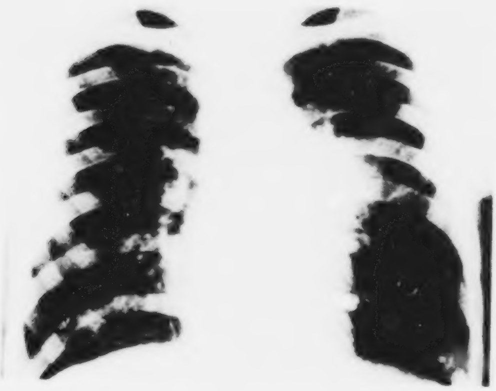
Once dissemination has occurred, the patient should be hospitalized for medical and surgical treatment. Careful nursing care is also extremely important.

Disseminating coccidioidomycosis (coccidioidal granuloma) was well described by Rixford, Gilchrist,(16) and Ophüls(17) in 1896 and 1905, and was recognized long before the primary phase of the disease process was understood. In 1937, the relationship between the primary coccidioidomycosis (Valley Fever) and coccidioidal granuloma was clarified by Dickson and Gifford.(18) Between 50 and 60 percent of the cases in which dissemination occurs end fatally.

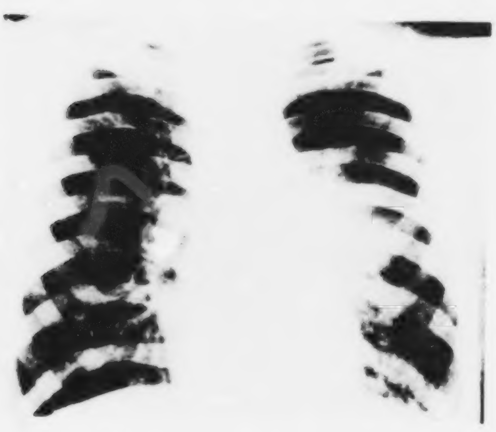
Death may occur after 4 to 6 weeks in acute fulminating cases, or not until many months later in more chronic forms of the disease. It is now generally accepted that disseminating infection is an entirely endogenous process which takes place as a continuation of the initial infection. C. E. Smith has stated that "reinfection is not from without, but from within, usually early after the primary infection. Only rarely does dissemination occur after a number of years, although occasional cases have been reported. These are exceptions which prove the rule."

During the dissemination process, X-rays will usually reveal a progressive pulmonary lesion, often accompanied by hilar or mediastinal adenopathy. Lymphadenitis may also appear elsewhere, following extension through lymphatic channels, leading to secondary abscess formation of distant lymph nodes which suppurate and drain through sinus tracts. With deeper involvement, mediastinal and retroperitoneal abscesses may result. The lungs may become involved by miliary infiltrate, resembling miliary tuberculosis, but more chronic in its progress. Dense pulmonary areas of consolidation sometimes appear, and pulmonary abscesses which can extend directly to the pleural space may form, then rupture and produce coccidioidal empyema. Continued extension produces involvement of overlying thoracic skeletal structures, resulting in destructive lesions of the ribs, sternum, vertebrae, scapulae, and clavicles and secondary abscess formation in the adjacent soft tissues of the chest wall. Extension to the pericardium and myocardium is not unusual. The solid abdominal viscera may become the seat of metastatic abscesses which can rupture and cause secondary peritonitis. At autopsy, necrotic nodules are frequently found in the spleen, liver, and kidneys. Destruction of the suprarenal glands with Addisonian crisis and death has also been reported. Spread of the infection to the central nervous system results in coccidioidal meningitis, which is more common in children, and occurs twice as often in males as in females. Death in acute meningeal cases usually occurs by the third month, but in more chronic disease by the twelfth month. Secondary hydrocephalus often develops. A few very prolonged cases of chronic meningitis have been reported; in one patient, its duration was five years. Bone lesions are frequent,(19) and are commonly destructive in type with sclerosing lesions seen only occasionally. Periostitis may also be present. Coccidioidal osteitis seems to prefer the skeletal protuberances such as the malleoli of the tibia and fibula, the tuberosity of the tibia, and the femoral epicondyles. The wrist, fingers, and tarsal bones along with the ulna, radius, skull, vertebrae, and pelvis are frequently involved. This predilection for bony prominences also includes the ends of long bones. Both epiphyses and diaphyses may be infected and there may be direct extension into the joint space. Bone infection is often followed by secondary soft tissue abscesses and draining sinuses. In Forbus there are good descriptions of the pathology encountered in studies of 95 cases of dissemination.(20)

## DISSEMINATING COCCIDIOIDOMYCOSIS

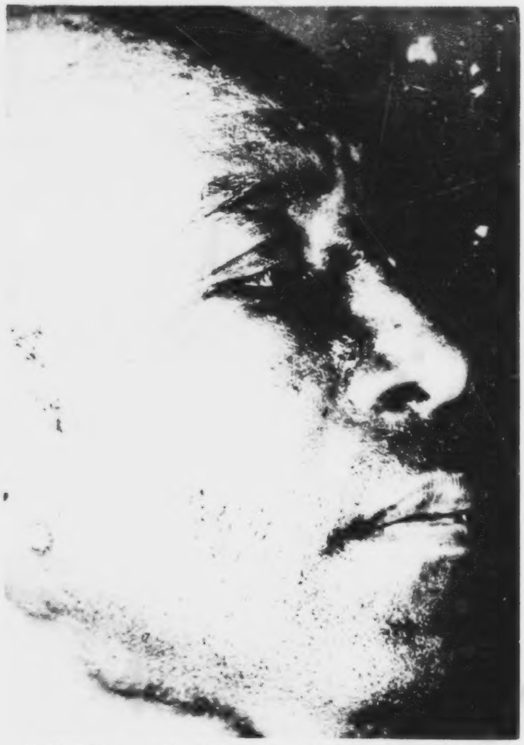


(D.G.) March 6, 1940. Marked lymphadenopathy, left hilum, associated with primary coccidioidal infection.



(D.G.) April 24, 1940. Same patient 6 weeks later, showing extension of disease to left base. (Such adenopathy in the hilar or paratracheal area is of ominous significance. In this patient, death occurred 16 weeks after onset.)

Many preparations and compounds have been used in an attempt to control dissemination, but none as yet are fully proven. Surgical treatment includes drainage of abscesses, with removal or curettement of involved lymph nodes, excision of sinuses, and removal of necrotic bone. Localized amputation of the digits or extremities must sometimes be considered when there are destructive bone lesions. Cauterization of shallow skin ulcers or verrucous lesions with the high frequency current is extremely important and must be carried out and repeated as often as necessary. This is justified by the concept that all distant foci are metastatic in origin, and that they in turn metastasize, compounding the sources of spherule origin until the tissues are literally overwhelmed by these rapidly self-producing foreign bodies. One must also include the drainage of empyema. In coccidioidal meningitis, the frequent, almost daily drainage or even replacement of infected cerebrospinal fluid is indicated. The prevention of hydrocephalus may require such procedures as the Torkelson operation, although its ultimate value is questionable.



(J.U.) Two metastatic skin lesions of coccidioidal type, on right side of nose and below right ear in a forty-six-year-old Negro, Such lesions should be destroyed by cauterization with the high frequency current. In this patient, death occurred three and one-half months after admission.

The need for a specific agent that could be used during the primary phase of infection remains urgent. If this substance is not fungicidal, it should at least be fungistatic in order to prevent the dissemination process. Aminostilbamidine, which seems to have a definite suppressive effect, is under study at the present time. Amphotericin B shows promise, but a recently introduced intravenous form, which to date is untried, may be of greater value.

The pulmonary sequelae of primary coccidioidal infection of the lungs form a group of interesting static changes that arise in about 5 percent of the patients. Such lesions have been fully described in the literature,(21) with Farness first reporting cavitation in 1939.(22) As stressed in a recent review by Hensler and Cleve,(23) these chronic residual pulmonary changes require careful study to avoid confusing them with tuberculosis and with neoplastic disease.

From the clinical standpoint, the most important residual pulmonary lesions are the cyst-like cavity, the soft-solid focus (coccidioidoma), and the abscessing coccidioidoma. Residual calcified pulmonary foci, including calcified lymph nodes, are common endpoint lesions, but have little clinical significance. They should be differentiated from tuberculous and histoplasmotic calcifications.

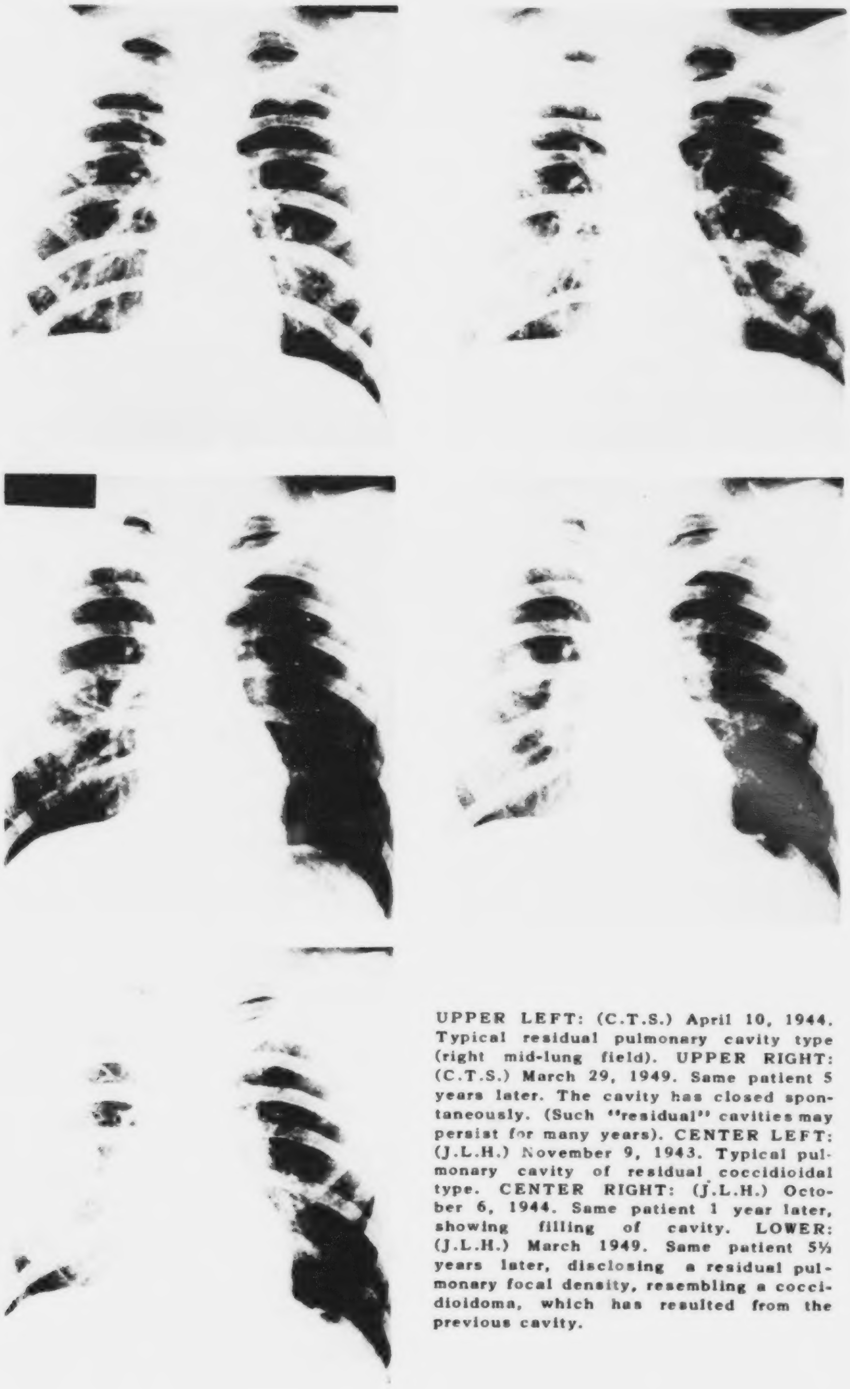
The correct therapeutic approach toward this group of residual pathologic changes in the lung will depend on the recognition of their coccidioidal origin and an understanding of their development and potentialities.

The choice between medical management and surgical extirpation should be based upon a definite set of criteria, and adherence to proper conservatism. Effective chemical "coverage" such as is available in the surgical treatment of pulmonary tuberculosis is lacking in this disease; hence serious complications are not unusual following surgical treatment of these pulmonary coccidioidal lesions. It is generally agreed that the existence of these lesions, *per se*, does not indicate any activity of the coccidioidal infection. In only rare instances has progressive disease resulted from residual cavitation or coccidioidoma.

The typical residual pulmonary cavity is thin-walled and cyst-like in appearance (24) and often harbors the spherules of *C. immitis*, or occasionally the mycelial elements. The presence of residual cavities apparently confers an immunity solid and lasting enough to protect the host against additional inroads by the fungus. The mechanism may be similar to the one which permanently protects a person who recovered from coccidioidomycosis against exogenous reinfection.

Our impressions, based on the careful observation of 200 cases of residual pulmonary cavitation seen over the past 15 years, indicate that:

1. Intermittent hemoptysis can be expected in about 65 percent of the cases. In our group, there were 2 known fatalities as a result of hemorrhage.
2. Cavities close spontaneously in 25 percent of the cases. In approximately 50 of our patients, cavities ultimately measured less than 2 cm. in diameter.
3. Cavitation can persist unchanged for many years with no evident harm to the patient. This was observed in approximately 20 (10 percent) of our patients.



UPPER LEFT: (C.T.S.) April 10, 1944. Typical residual pulmonary cavity type (right mid-lung field). UPPER RIGHT: (C.T.S.) March 29, 1949. Same patient 5 years later. The cavity has closed spontaneously. (Such "residual" cavities may persist for many years). CENTER LEFT: (J.L.H.) November 9, 1943. Typical pulmonary cavity of residual coccidioidal type. CENTER RIGHT: (J.L.H.) October 6, 1944. Same patient 1 year later, showing filling of cavity. LOWER: (J.L.H.) March 1949. Same patient 5½ years later, disclosing a residual pulmonary focal density, resembling a coccidioidoma, which has resulted from the previous cavity.

4. Cavities may fill with fluid and form an unchanging solid focus (blocked cavity), as was noted in 6 percent of our patients.
5. Transpleural rupture can occur, causing spontaneous pneumothorax and, occasionally, coccidioidal empyema. This happened in 2 percent of our cases.

Surgical removal of residual cavities becomes necessary when:

1. Repeated pulmonary hemorrhage occurs
2. The cavity continues to enlarge
3. A peripheral location threatens transpleural rupture
4. A residual cavity becomes secondarily infected and "balloons"
5. The coccidioidoma softens and abscesses.

Occasionally, in dealing with thin-walled residual cavities that are between 2 and 3 cm. in diameter and not too near the pleural surface (danger of rupture), we still use carefully controlled pneumotherapy, giving either pneumothorax or pneumoperitoneum. Such measures have resulted in closed cavities in 36 patients and have failed in 14.

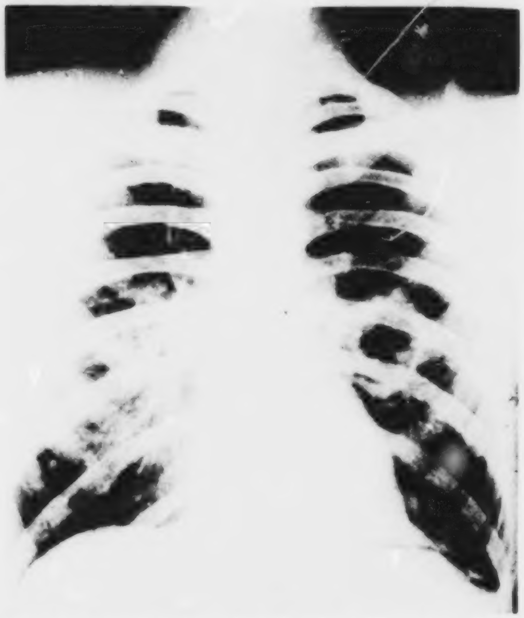
Surgical treatment, usually segmental resection or lobectomy, was required in 64 patients, or in about a third of our group of 200 who had cyst-like residual cavities. In three instances we have used extrapleural thoracoplasty to close very large apical cavities.

Thick-walled cavities, usually the result of central softening of a coccidioidoma, in most instances require surgical removal because they are essentially chronic abscesses.

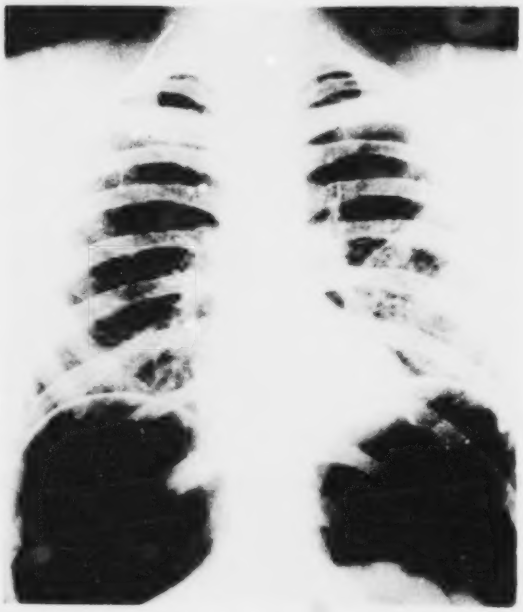
Excisional surgery should be wide enough to avoid cutting into diseased tissue and meticulous care should be used by the surgeon to prevent soiling or contamination of the pleural space by *C. immitis*. Lobectomy may, therefore, come to be the procedure of choice when cavities measure 3 to 4 cm. or more in diameter. Prompt obliteration of remaining pleural space is necessary after pulmonary resection, and will often necessitate elevation of the diaphragm by pneumoperitoneum for several months. This latter procedure will also safeguard against over-stretching of the remaining lung tissue – a contingency which may have something to do with the development of recurrent coccidioidal cavities appearing in the remaining lung following resection.(25) There were three such cases in our series.

Coccidioidomas fall into the group of "coin lesions," and are found fairly often as residuals to previous primary coccidioidomycosis. As has been

**PNEUMOTHERAPY (Pneumoperitoneum)**

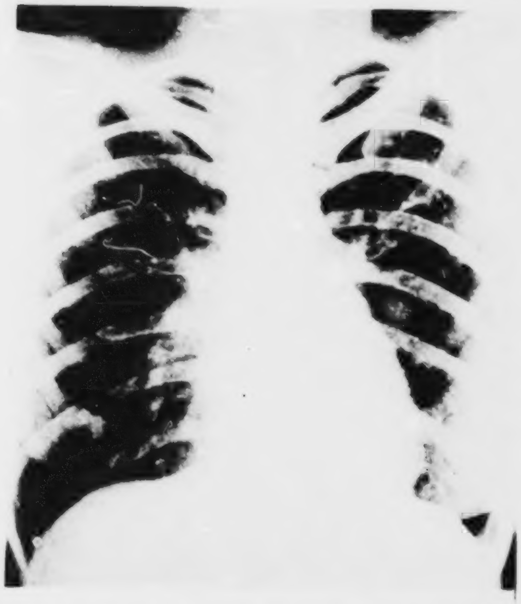


(C.C.) February 25, 1949. A large residual coccidioidal cavity near the left hilum.

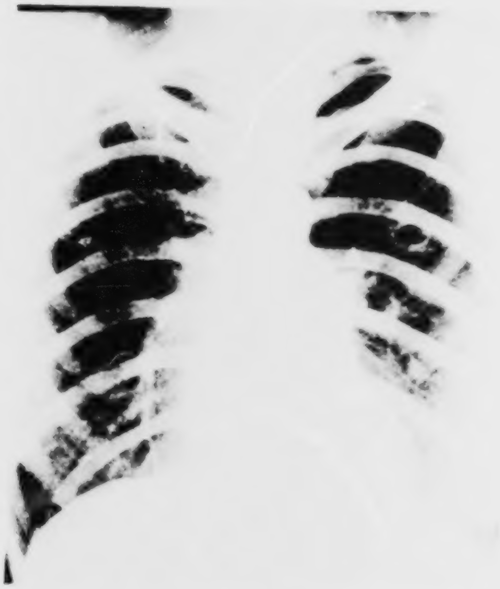


(C.C.) November 25, 1949. Pneumoperitoneum in effect 9 months, disclosing closure of the cavity. (Patient last seen August 25, 1956 and X-ray revealed no evidence of previous cavitation.)

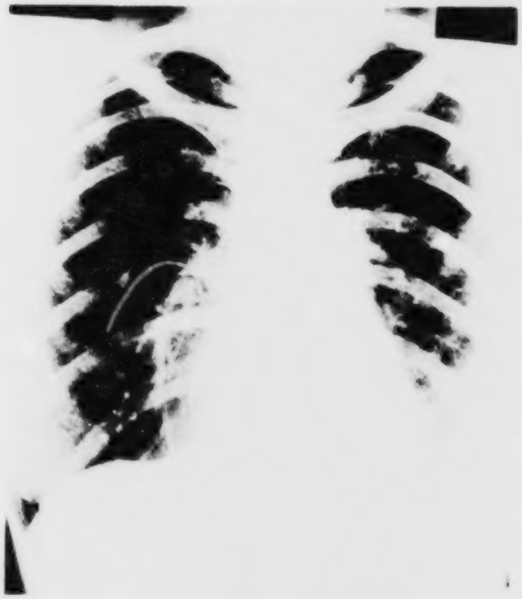
## PNEUMOTHERAPY (Pneumothorax)



(B.P.) March 14, 1949. Typical residual cavity in left upper lobe.

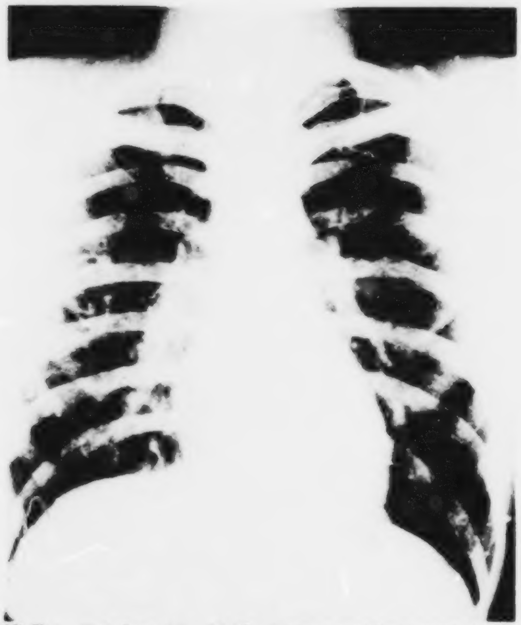


(B.P.) May 3, 1949. Same patient 2 months later, under artificial pneumothorax therapy cavity is smaller.

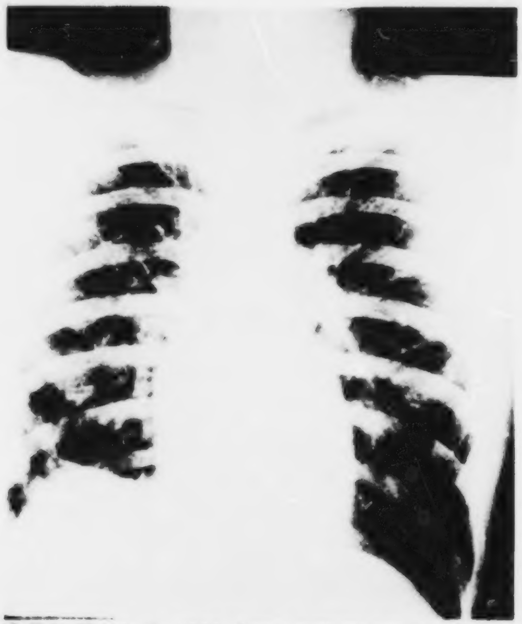


(B.P.) July 22, 1952. Same patient over 3 years later; lung re-expanded, cavity closed, with only a small nodular density remaining at first interspace.

## SIMPLE EXCISION

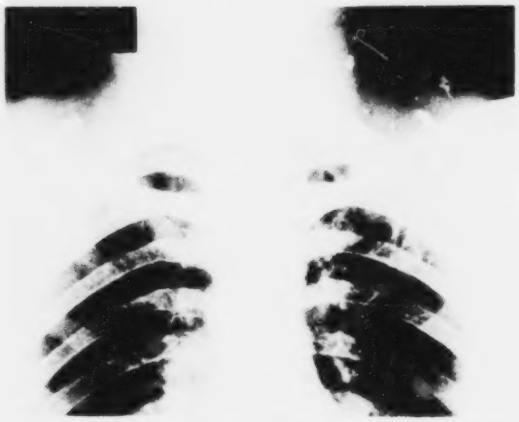


(O.T.) October 16, 1944. Large residual coccidioidal cavity on the right.

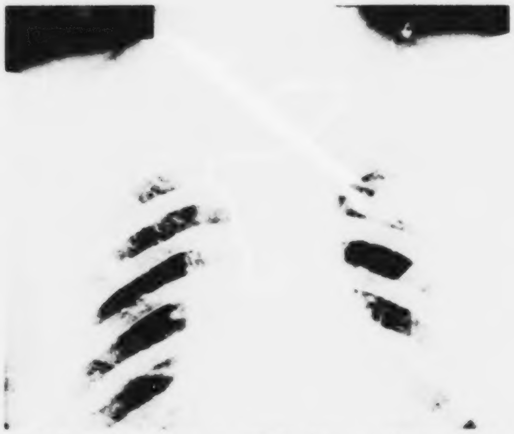


(O.T.) March 7, 1949. Same patient 4 years after removal of cavity by simple surgical excision.

## LOBECTOMY



(C.F.S.) January 10, 1949. Large coccidioidal cavity occupying left apex.



(C.F.S.) November 1, 1952. Same patient almost 4 years later, showing complete eradication following left upper lobectomy in January 1949.

stated, they occasionally result from "filling" of a residual, "blocked" cavity and persist thereafter as a solid appearing focus. Single or multiple coccidioidomas may develop, but in every instance, they closely simulate pulmonary malignancy. Within endemic areas, they are frequently discovered in minifilms taken during chest X-ray surveys. In the southern San Joaquin Valley, for example, the coccidioidoma so dilutes the incidence of malignant coin lesions that less than 0.5 percent of the entire group prove to be due to tumor. In case of doubtful diagnosis, their surgical removal and direct study is always advisable.

Melick(26) has summarized the opinions of a group of thoracic surgeons concerning excisional surgery of coccidioidal pulmonary lesions. I know that he has found divergence of opinion, with about 50 percent favoring a conservative approach because of the few but relatively serious complications that sometimes follow excisional surgery.

The eventual solution of the many problems pertaining to coccidioidal disease will come from a mutual exchange of ideas between laboratory and field research teams, public health officials, and clinicians. The need for an effective, specific control of the primary infection sufficient to prevent dissemination is paramount. Effective management and specific treatment of the disseminating phase is also required. As long as residual pulmonary lesions appear, clearly defined indications for surgical treatment will be necessary. These should be based only on conclusions derived from careful preoperative study and adequate follow-up of many patients.

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# The Viability of the Tissue Phase of *Coccidioides immitis*

Donald B. Louria, M.D., Ned Feder, M.D., and Chester W. Emmons, Ph.D.

The viability of the saprophytic stage of *Coccidioides immitis* has recently been investigated thoroughly by Friedman and her co-workers.(1) In this preliminary discussion we have attempted to define the duration of survival of the parasitic or tissue phase of *C. immitis* under various conditions of temperature and humidity. The study was made in two parts: the first utilized pus from subcutaneous abscesses in a patient with disseminated coccidioidomycosis; and the second employed suspensions of ground viscera from mice experimentally infected with *C. immitis*. It is emphasized that this report represents a partially completed project. Consequently, our conclusions may well have to be revised as more data become available.

## I. The Effect of Drying on the Viability of Spherules from Subcutaneous Abscesses.

**Method and Materials:** Pus was obtained by aspiration of subcutaneous abscesses of a 21-year old patient with far advanced disseminated coccidioidomycosis (National Institutes of Health, Clinical Center, Patient No. 11724). All direct cultures of this pus on Sabouraud's agar slants at 30° C. had many colonies of *C. immitis* after three to four days, with arthrospores developing in four to seven days. Each drop of pus contained from 10 to 25 typical spherules of *C. immitis*, many of which contained endospores.

The pus was drawn into a one-milliliter pipette and a drop placed on each of 32 autoclaved strips of filter paper, each strip measuring approximately one by one centimeter. Sixteen of the strips were placed in each of two sterile Petri dishes, which were covered and kept at room temperature, 22 to 25° C. No attempt was made to control the humidity of the room. At intervals of 24 hours at first, then later of two to three days, two strips were removed from a Petri dish. One strip was placed face down on a Sabouraud's agar slant, drawn back and forth over the slant several times, and left on the slant. Slants were then incubated at 30° C., and all negative cultures were held for 30 days before they were discarded. The second strip was placed in 4 ml. of isotonic saline and thoroughly shaken. One ml. of the saline was then inoculated intraperitoneally into each of four general-purpose white mice. One month later the mice were sacrificed and the liver and spleen cultured on Sabouraud's agar slants at 30° C.; again all cultures were held for 30 days before being considered negative.

**Results**: Strips with dried pus remained positive on direct culture in all specimens from the first to the thirty-fourth day. For the first 16 days more than one colony appeared on the Sabouraud's slant, whereas thereafter only one colony was present, indicating some decrease in the number of viable spherules remaining on the dried strip. Those spherules that survived seemed to have unimpaired viability, since mycelia and arthrospores appeared just as quickly on culture after 34 days of drying as on the first day.

After 35 days of drying none of the dried filter paper strips showed any growth of *C. immitis* when placed on Sabouraud's agar slants.

All the mice inoculated with saline suspensions of dried pus had negative hepatic and splenic cultures with a single exception. Of four mice inoculated with a saline suspension of pus from a filter paper strip dried 10 days, one had positive hepatic and splenic cultures.

Thus, under these conditions of drying at room temperature, the pus contained viable spherules after 34 days as determined by direct growth on Sabouraud's agar and after 10 days as determined by mouse inoculation.

## II. Survival of Spherules of *C. immitis* at Various Temperatures and Humidities.

**Method and Materials:** Spherule-containing material was obtained from 17 to 20 gram general-purpose white mice experimentally infected with

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*From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.*

*C. immitis* subcultured from the pus used in Part I. A saline suspension containing approximately one thousand arthrospores of *C. immitis*, harvested from a 3-week culture on Sabouraud's agar at 22 to 25° C., was injected intravenously into each mouse via a lateral tail vein. Mice were sacrificed when moribund, which was within seven days after inoculation. Liver and lung were ground in a mortar, emulsified in saline, and filtered through a cotton pad. On microscopic examination of the suspension, there were 2 to 6 spherules in each low-power field.

A drop of the suspension was placed on a strip of filter paper as described in Part I, and 15 such strips were placed in each of 16 Petri dishes. The Petri dishes were placed under various conditions of humidity and temperature.

The method of controlling humidity was similar to that of Friedman *et al.*(1) Petri dishes were placed in dessicators or sealed jars, the bottom of which were filled with appropriate solutions. Relative humidities of 30 to 90 percent were obtained with solutions of calcium chloride; of 10 percent with a saturated solution of lithium chloride with excess solid lithium chloride; of 0 percent with anhydrous calcium sulfate; and of 100 percent with water.(2, 3)

The conditions studied were 0, 10, 30, 50, 70, 90, and 100 percent relative humidity at 22 to 25° C. (room temperature). At 4° C. and 37° C., utilizing a similar technique, Petri dishes were kept at 0, 50, and 100 percent relative humidity.

A 4-cm. circular hole was cut in the top of each Petri dish, and the hole covered with filter paper affixed with tape. It was considered that by this means the interior humidity of the Petri dish would equilibrate more rapidly with the outside humidity.

At each of the three temperatures, one Petri dish was exposed to room air without any attempt to control humidity. Finally, five ml. of the spherule-containing suspension were placed in each of two small flasks, one kept at 22 to 25°, the other at 37° C., to determine the survival of spherules in liquid suspension at the two temperatures.

At intervals of 24 hours, then of 2 days, and finally of 3 days, a strip from each of the Petri dishes was placed on Sabouraud's agar slants and incubated at 30° C. The final strip was transferred after 32 days of exposure.

**Results**: The results are depicted in table 1. Several features may be noted. Viability of the spherule at 4° C. was greatly decreased. At this temperature at 0 percent relative humidity, spherules were viable for only 24 hours, while at 50 percent relative humidity, cultures were negative after three days of drying with the single exception of one positive culture on the eleventh day. At 100 percent relative humidity, all cultures were negative after the thirteenth day with the single exception of one positive on the thirty-second day. It is evident that at this temperature as a rule, spherule survival was greatly diminished at all three humidities. However, the single positive culture at 100 percent humidity on the thirty-second day after negative cultures for the preceding 19 days, indicates that survival of spherules may occur even under these unfavorable conditions of low temperature.

At both 37° C. and room temperature survival was considerably better at humidities of 0 to 30 percent and 90 to 100 percent than at humidities of 50 to 70 percent. Thus at room temperature, humidities from 0 to 30 percent and 90 to 100 percent yielded for the most part positive cultural results throughout the 32-day experimental period, whereas at 50 percent and 70 percent humidities, no positive cultures were obtained after the thirteenth day. At 37° C., positive cultures were obtained at 0 percent and 100 percent throughout the 32-day period, while at 50 percent humidity strips were negative after the third day of drying.

At both room temperature and 37° C., mycelia grew directly on the filter paper strips during their exposure to humidities of 90 to 100 percent. Mycelia were noted after the fourth day, producing a small number of arthrospores by the sixth day. This obviously represents very early conversion to the mycelial phase without an intervening period of dessication.

Spherules dried at room humidity again demonstrated the deleterious effect of low temperatures; no positive cultures were obtained after three days of drying at 4° C. On drying at 37° C., 24-day survival was noted, while at room temperature and room humidity positive cultures were obtained throughout the 32-day period.

Finally, it was noted that spherules in liquid suspension survived considerably longer at room temperature than at 37° C. The viability at room temperature extended throughout the 32-day experimental period, whereas at 37° C., no positive cultures were obtained after 15 days.

It appeared then that under these experimental conditions survival of dried spherules was significantly diminished by either of two factors: low temperatures (4° C.) or intermediate relative humidities (50 to 70 percent). Moreover, when spher-

## TABLE 1

### SURVIVAL OF SPHERULES UNDER VARIOUS CONDITIONS OF TEMPERATURE AND HUMIDITY

| TEMPERATURE | RELATIVE HUMIDITY | DURATION OF SURVIVAL IN DAYS * |
|---|---|---|
| 4° C | 0 | 1 |
| | 50 | 11 |
| | 100 | 32 |
| | Room ** | 3 |
| 22 – 25° C | 0 | 18 |
| | 10 | 32 |
| | 30 | 32 |
| | 50 | 11 |
| | 70 | 13 |
| | 90 | 32 |
| | 100 | 32 |
| | Room ** | 32 |
| | Liquid | 32 |
| 37° C | 0 | 28 |
| | 50 | 3 |
| | 100 | 32 |
| | Room ** | 24 |
| | Liquid | 15 |

* Maximum survival observed was 32 days at which time the experiment was terminated.

** Strips were exposed to room humidity which was not controlled.

ules were maintained under conditions of high relative humidities (90 to 100 percent) at moderate temperatures (22 to 25° and 37° C.), mycelia appeared within four days and arthrospores within six days.

## Discussion

Rosenthal and Routien⁽⁴⁾ noted that spherules remained viable after 110 days at 12° C. Rosenthal and Elmore⁽⁵⁾ also reported that spherule-containing sputum which had been kept in the shade for 136 days produced pathological lesions when injected intraperitoneally into guinea pigs; and *C. immitis* was cultured from these lesions.

Hardiness of the parasitic phase of *C. immitis* is confirmed in the present report. Only low temperatures and intermediate humidities prevented survival of some spherules. At higher temperatures (22 to 37° C.), under conditions of low (0 to 30 percent) or high (90 to 100 percent) relative humidity, spherules survived throughout the experimental period. They might have survived considerably longer if the experiment had not been terminated.

It is interesting to compare these results with those of Friedman and her associates.⁽¹⁾ Working with the saprophytic phase of *C. immitis*, they observed that low humidity (10 percent) was unfavorable to spore survival, in contrast to our finding that intermediate humidities shortened spherule survival. Friedman *et al.*(1) also reported high temperatures (37° C.) to be injurious to spore viability, whereas it was noted in the present study that low temperatures decreased spherule survival.

These differences may represent actual differences between the parasitic and saprophytic phases of *C. immitis* in survival ability under various conditions of humidity and temperature. It is also possible that these differences are due to dissimilarities in the strains of *C. immitis* employed.

There were some similarities in the two studies. Both spores and spherules, kept moist in physiologic saline, survived considerably longer at room temperature than at 37° C.

Although the present study has demonstrated prolonged spherule survival under various conditions of temperature and humidity, it is questionable whether this sheds any light on the problem of contagion. Most authors, including Smith,(6) Baum,(7) and Fiese *et al.*,(8) Bass *et al.*,(9) and Jacobson(10) feel that there is no evidence for man-to-man transmission of coccidioidomycosis, and no significant danger of such contagion. Rosenthal and Routien,(11) however, noted positive skin tests and pathological lesions compatible with coccidioidomycosis in normal guinea pigs that were kept in the same cage with guinea pigs infected with *C. immitis*. This, of course, does not necessarily indicate direct animal-to-animal transmission, since it does not rule out the intervention of mycelial growth with arthrospore formation from fomites in the cage. These workers also noted that pulmonary infection could be produced by intratracheal instillation of spherules into guinea pigs. On the basis of this experimental evidence, it is conceivable that direct man-to-man transmission of the parasitic phase of *C. immitis* could occur. In nonendemic areas this possibility should be considered in the hospitalization of patients discharging viable spherules in sputum or pus, even though direct man-to-man transmission has not been proved. This concept derives some support from the demonstration of hyphal elements under parasitic conditions as reported by several investigators and as discussed in a recent review by Fiese *et al.*(8)

In view of the ability of spherules to survive and to form mycelia and arthrospores even under adverse conditions, it may also be important to prevent mycelial growth and arthrospore formation by frequent changes of dressings, immediate disinfection or incineration of all dressings and fomites, and regular and terminal cleaning and disinfection of walls, floors, and bedding exposed to patients discharging viable *C. immitis*.

## Summary

The survival of spherules of *C. immitis* under various conditions of temperature and humidity has been investigated. It is noted that the spherules remained viable for long periods of time (32 to 34 days) at temperatures from 22 to 37° C., and at relative humidities from 0 to 30 percent and 90 to 100 percent. Low temperature, namely 4° C., and intermediate relative humidities of 50 percent and 70 percent were found to be unfavorable to spherule survival.

Spherules kept in physiologic saline showed considerably longer survival at room temperature than at 37° C.

The difference and similarity between spherules and spores of *C. immitis* in regard to viability under various conditions of temperature and humidity are noted. Finally, it is felt that in the light of the present experimental evidence, contagion, no matter how unlikely, is possible, and it is suggested that this possibility be considered in the care of patients with spherule-containing fomites.

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# Survival of the Pathogenic Fungus *Coccidioides immitis* Under $CO_2$ Refrigeration

**William L. Jellison, Ph.D., and Hazel Parker, M.A.**

The examination of wild rodents for infection with *Coccidioides immitis* is one of several methods of detecting endemic areas of coccidioidomycosis. Other methods include diagnosis of acute and chronic cases of the disease in resident human population, skin-testing humans and cattle with coccidioidin antigen, examination of cattle for evidence of infection at time of slaughter, and testing soil samples either by direct culture or inoculation into susceptible animals. Each of these methods has definite advantages and disadvantages, but the examination of wild rodents for infection appears to be one of the more practical procedures.

Examination of rodents in the field with makeshift laboratory facilities is possible but often unsatisfactory; therefore, the preservation of animals for later examination in adequately equipped laboratories poses a problem. $CO_2$ refrigeration of rodents at the time of collection would solve this problem provided the infectious agent could survive such refrigeration. $CO_2$ refrigeration temperatures are approximately -60° to -70° C. The following experiment was designed to test the survival of *C. immitis* under such conditions.

An experimentally infected mouse which was autopsied on August 24, 1954, showed extensive lesions characteristic of *C. immitis* infection. Two series of cultures were prepared from this mouse on Sabouraud's medium in test tubes sealed with screw tops. In one series, bits of infected spleen were used for inoculum, and in the other a suspension of spherules, teased from an abscess in the mouse, served as inoculum.

One pair of tubes was incubated immediately at room temperature to check the viability of the inocula. The other tubes were wrapped in cellophane bags and placed in a $CO_2$ refrigeration unit. At intervals shown in table 1, paired tubes were removed from refrigeration and incubated at room temperature. It was assumed that this exposure to low temperatures would be comparable to, or at least as severe as, that experienced by organisms within a rodent when placed in $CO_2$ refrigeration. The results of this test are presented in table 1.

One or both cultures of each pair showed mycelial growth typical of *C. immitis* after periods of storage from 1 to 340 days in a $CO_2$ refrigeration unit. In each of the 12 tests, the suspension of spherules remained viable. Growth occurred in 8 of the 12 tubes in which a portion of spleen was used for inoculum. It is not certain that all portions of the spleen contained spherules. One culture with spleen inoculum showed growth after 202 days' storage.

The two cultures removed from storage after 202 days' refrigeration were each used, after mycelial growth appeared, as inoculum for 8 mice. At necropsy all 8 mice exhibited gross lesions characteristic of *C. immitis* infection and spherules were demonstrated in the lesions. Growth from spherules after 340 days' refrigeration was likewise tested for infectivity by injection into mice. Typical infection resulted.

## Summary

Spherules of *C. immitis* from experimentally infected mice, and the organism in bits of spleen, remained viable under $CO_2$ refrigeration for 202 days. One tube of spherules was viable after 340 days when the experiment was terminated.

It is concluded that wild rodents preserved by $CO_2$ refrigeration at the time of collection would be suitable for examination for *C. immitis* after several months' storage. Some loss of viability might be experienced after the first 7 days.

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*From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana.*

## TABLE 1

## SURVIVAL OF *C. IMMITIS* AFTER STORAGE AT LOW TEMPERATURE

| Days Storage | Growth from Spherule Suspension | Growth from Spleen Fragment |
|---|---|---|
| 1 | + | + |
| 2 | + | + |
| 3 | + | + |
| 7 | + | + |
| 14 | + | − |
| 21 | + | − |
| 28 | + | + |
| 97 | + | + |
| 126 | + | + |
| 174 | + | − |
| 202 | + (infective) | + (infective) |
| 340 | + (infective) | − |

+ = Growth of *C. immitis*

- = No Growth

# Coccidioidal Bone Infections

J. R. Schwartzmann, M.D.

One of the more common groups of lesions encountered in disseminated coccidioidomycosis involves bones and joints. Various authors estimated that from 1:500 to 1:1000 cases of primary infection will progress to the secondary, disseminated, or granulomatous phase of the disease. Approximately 20 percent of these disseminated cases involve bones and joints.

The mortality rate in disseminated coccidioidomycosis – whether bony or systemic – is approximately 50 percent or higher. The prognosis is less favorable in patients of the dark-skinned races than in white people. There is no well-organized, complete monograph detailing coccidioidal bone infection in the literature, but consistent findings which adequately describe this phase of the disease have been accumulated from numerous reports of bone and joint involvement.

### Pathogenesis and Pathology

Bone and joint lesions result from dissemination of the spores through the blood stream which produces active foci, particularly in cancellous or spongy bone, and affects adjacent soft tissue structures simultaneously. Synovial and tendon sheath infections, without demonstrable underlying bony infections, are reported; however, these apparently occur less frequently than direct bone infection.

Grossly, the bone and joint lesions are characteristic only of inflammatory or infectious disease. They are marked by redness, swelling, local heat, and at times fluctuance or abscess formation, or non-specific purulent drainage. Chronicity of drainage, failure of lesions to heal, and indolent ulceration in this disease are closely akin to similar symptoms found in tuberculosis. Primarily, bone destruction and lysis are noted when lesions are examined grossly.

Under the microscope coccidioidal osteomyelitis specimens have a granulomatous, non-specific appearance with round cell and polymorphonuclear infiltration, simple cellular necrosis, giant cells, and epithelioid tubercle formation. Specific diagnosis by microscopic examination depends upon demonstrating the specific refractile, double-walled spherule (fig. 1). Purulent exudate from draining sinuses may reveal the presence of the spores – and these may be demonstrated as present by proper culture technique.

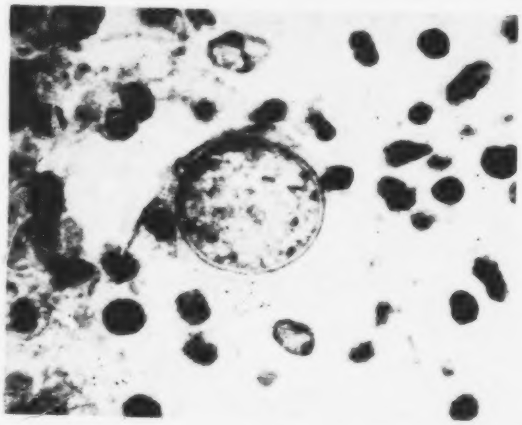

Figure 1. Microscopic appearance of the thick-walled sporangium of *Coccidioides immitis*.

### Diagnostic and Clinical Features

The ultimate diagnosis of coccidioidal bone infection is dependent upon the demonstration of the causative organism microscopically, in histologic specimens, or upon isolation of the organism from tissue or exudate. The presence of coccidioidal bone lesions may be unsuspected for long periods because early signs and symptoms are not specific.

In regions endemic for coccidioidomycosis, differential diagnosis must include the possibility of coccidioidal infection in persons with persistent vague complaints and those involving the bones and joints. During some periods of observation it may be nearly impossible to eliminate tuberculosis, since the clinical manifestations of coccidioido-

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mycosis and tuberculosis may parallel each other.

Of diagnostic value from a clinical standpoint are the following observations:

1. A history of previous primary coccidioidal infection, or a history of possible exposure in endemic areas.

2. Complaints of persistent and prolonged joint pain and deep bone pain and aching or even acute and precipitous onset of such pain and aching.

3. Swelling, redness, local heat over bones, joints or tendons, with joint effusion in thickening, induration, and edema.

4. Fever – either low grade and persistent, or spiking and vacillating – with or without chills.

5. Painful limitation of joint motion, fluctuance, abscess formation, chronic drainage, and indolent ulceration are common in the course of the disease.

6. Chronic loss of weight, anorexia, malaise, and fatigue frequently accompany bone infection from coccidioidomycosis.

7. Specific laboratory studies.
    a) Culture of the fungus from tissue, joint fluid, and exudate or pus.
    b) Biopsy and microscopic demonstration of the sporangia.

8. Laboratory studies of a non-specific nature. Recently, a study of readily available case records of coccidioidal bone infections was reported to the Western Orthopedic Association. This study embraced cases from the records of orthopedists in Phoenix and Tucson. Interesting correlations of common laboratory work were made, in which the sedimentation rate, white blood cell differential count, urinalysis, and temperature curve recordings were made. In this series of cases, eosinophilia, albuminuria, and high sedimentation rate – often out of proportion to the patient's illness – were noted with great frequency. Eosinophilia of 5 or more was present in 7 of 16 cases where differential blood counts were recorded. In 7 of the 12 cases with urinalysis recorded, there was albuminuria ranging from a trace to 4 plus. In 8 of the 13 cases where the sedimentation rate was recorded, it was over 30 mm. per hr. and persistently elevated. The outcome is known in 12 cases in which the differential blood counts were recorded. Of the six patients with 5 or more eosinophiles, 5 died; the eosinophile count of the single survivor was 5. All 7 of the patients with eosinophilia below 5 lived. In 11 cases the sedimentation rate was recorded and the outcome is known. The three patients that died had rates of 85, 130, and 140. Of those who lived, one had a sedimentation rate of 116, one had a rate of 36, and the remainder had sedimentation rates of 30 or less.

In comparing the temperature curves against the final outcome, it was found that the eight patients whose highest recorded temperature did not exceed 100° F. recovered, whereas six patients with temperatures of 101° F. or more died. One patient whose highest recorded temperature was 104° F. survived.

9. Certain types of exudate obtained by incision and drainage of abscesses should immediately arouse suspicion of coccidioidal infection. This is true of an exudate that resembles tuberculous pus or one that is thin and watery, contains lumps and shreds of fibrin and necrotic soft tissue, and shows none of the common pyogenic bacteria on freshly made smears.

10. X-ray examination is of extreme importance diagnostically, although roentgen appearance is in no way specifically diagnostic. Reported observations in the literature indicate that coccidioidal lesions appear in cancellous or spongy bone, in bony prominences, processes, and tubercles, are characterized roentgenographically by simple bony destruction and lysis, tend to be cyst-like in appearance, and often show a tendency to proliferate subperiosteally. In a review of 22 cases of coccidioidal bone disease with 40 sites of infection, only one showed subperiosteal bone production. This was a 5-year-old child in whom the disease was rapidly fulminating and fatal (figs. 2a and b). Serial X-rays may show extremely variable pictures as the disease progresses with minimal to marked progression of osteolysis in varying temporal spans (figs. 3a, b, and c).

11. Biologic sensitivity tests such as the coccidioidin skin test have about the same diagnostic significance for coccidioidomycosis as tuberculin tests have for tuberculosis. The complement-fixation quantitative titer test, as performed by Dr. Smith at the University of California, affords positive diagnosis of active coccidioidal infection and may be of prognostic value when there are bone lesions in this disease.

Differential diagnosis is required to rule out tuberculous osteomyelitis, actinomycosis, blastomycosis, and other fungus granulomas. Acute bacterial osteomyelitis, rheumatic fever, rheumatoid arthritis, and the collagen diseases may resemble coccidioidal bone dissemination. Primary and metastatic bone neoplasms may require microscopic studies for differentiation.

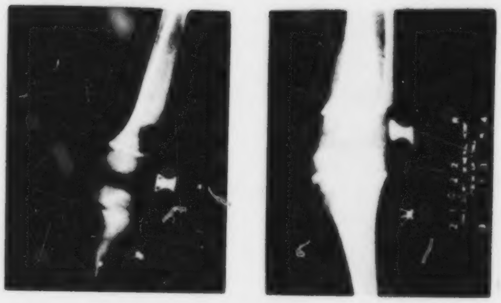


Figure 2a. Five-year-old child with rapidly fulminating coccidioidal osteomyelitis on admission to hospital.

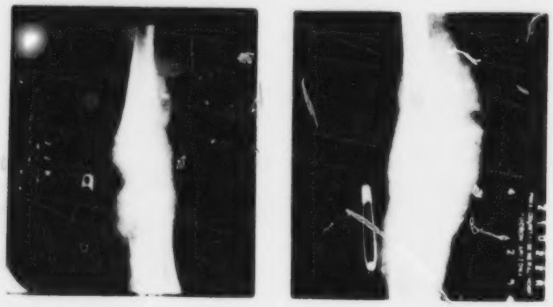


Figure 2b. Same child three weeks later showing subperiosteal reaction and markedly increased bone destruction.

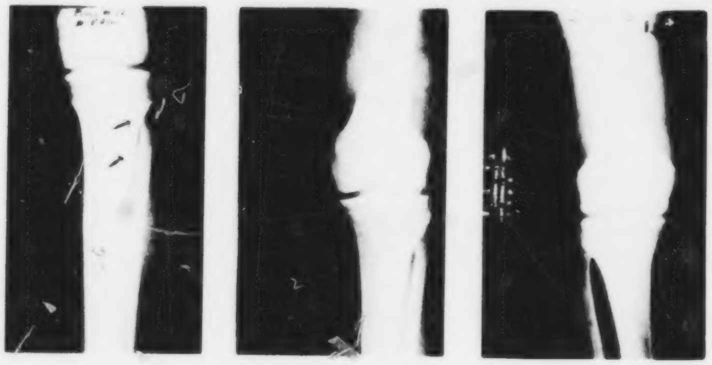


Figure 3a. X-rays spanning a period of four years show no apparent progression of destruction in tibial lesion in patient with active coccidioidal osteomyelitis.

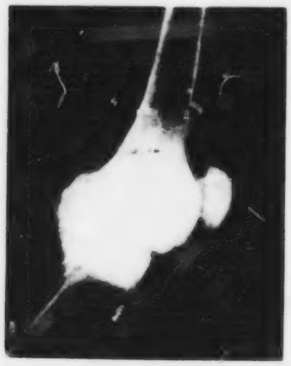


Figure 3b. The same knee after an additional five year span showing marked destruction.

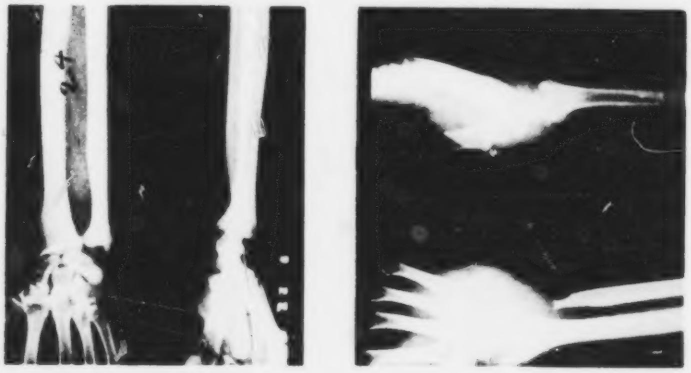


Figure 3c. Same patient showing evident destruction in wrist over a seven year span.

## Treatment

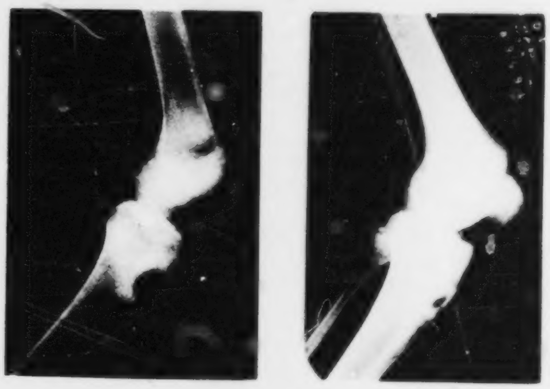
At present there is no effective therapy for disseminated coccidioidomycosis of the bones and and joints. Occasionally isolated reports of "cures" appear in the literature, but it has not been possible to repeat the results in the vast majority of cases.

Surgical excision of single lesions (figs. 4a and b) has offered apparent cure in selected cases. Amputation, local bone excision, incision and drainage, synovectomy, and radical tendon sheath excision have effected a small number of "cures." These measures, however, are limited in use and and are not effectively applicable to the over-all spectrum of coccidioidal osteomyelitis.

Of empiric value, orthopedically, are plaster casts, braces, and occasional surgical fusion of involved joints which are not actively infected.

## Conclusions

In conclusion, certain clinical features of coccidioidal bone infections and problems in diagnosis of coccidioidomycosis have been presented. There is no effective therapy, and the prognosis in disseminated coccidioidomycosis of the bone is most discouraging in the light of present knowledge.



Figures 4a and b. (a) Appearance of an isolated lesion in upper tibia treated by incision and drainage. (b) Same patient four years later, infection quiescent, no drainage, wound healed, laboratory findings normal.

# Diagnostic and Biologic Implications of the Histopathology of Coccidioidomycosis

Robert W. Huntington, Jr., M.D.

My colleagues and I have long been interested in the histopathology of human coccidioidomycosis as an approach to the nosology and biology of that disease. We had always intended to write about our observations in this field. The invitation to participate in this symposium catalyzed that intention into a determination to take the time to write such a paper. We accept this invitation with pleasure, and we are very grateful to the friends who invited us. We are the more pleased because we are convinced that the high hopes and the great accomplishments of the current serologic investigations and the syntheses of drugs have not lessened the usefulness of the histopathologic approach to the biology of coccidioidomycosis. We earnestly wish for better communication and cooperation between pathologists and public health workers. Leaders in public health such as C. E. Smith,(1) Myrnie Gifford,(2) and Libero Ajello, and such leaders in pathology as E. M. Butt(3) have contributed much to the study of coccidioidomycosis at Kern General Hospital.

I have not attempted to make a thorough review of the literature. Good bibliographies will be found appended to the papers by Alznauer, *et al.*,(4) and Forbus and Besterbreurtje.(5) In advance, I apologize to any author who may have expressed these ideas previously, and to those whose divergent opinion I have not given appropriate consideration.

I do not intend to compare the histopathologic, mycologic, immunologic, and epidemiologic approaches to the study of coccidioidomycosis. All of these approaches complement and do not rival one another. They are all useful. For example, in a case of coccidioidomycosis of the lungs with cavity, the Smith test(1,4) may be negative, and, whether or not the serologic reaction is positive, an attempt should be made to isolate the microorganism by sputum culture. If this should fail, the diagnosis could still be made with some assurance in a known endemic area, if the patient reacts to coccidioidin but is tuberculin-negative. The diagnosis may sometimes be confirmed by culture of *Coccidioides immitis* from a specimen from a resected cavity wall or by demonstration of spherules in sections of the specimen. There are some cavities, however, for which the etiology cannot be determined. In an endemic area, some of these are probably caused by *C. immitis.*

Since a rise in complement-fixing antibody titer is of grave prognostic significance, it is difficult to visualize this antibody as a protection against coccidioidomycosis. The results of investigations of other possible antibodies should prove interesting. It is possible that an explanation of the fact that most people infected with *C. immitis* localize and overcome the infection may be found in a study of tissue rather than of sera.

In our study of the histopathology of coccidioidomycosis, my colleagues and I have been concerned as much with the distribution and structure of the microorganism as with the pattern of the host response. We are primarily interested in the morphology of *C. immitis* in tissue, but that does not mean we are not interested also in the isolation of that fungus in culture. We agree with Weed(6) that the recognition of a pathogenic fungus by microscopic examination of the tissues is more satisfactory if it is confirmed by culture, and that cultures are sometimes convincing when direct microscopic detection is difficult or impossible. But cultures also have their limitations. They are, for example, sometimes negative in instances of cavitation.(7) We have repeatedly failed to obtain positive cultures from localized fibrocaseous lesions without cavitation, even though the tissue itself was cultured and typical spherules could readily be detected in tissue sections. Material from disseminated coccidioidomycosis which contains recognizable spherules will usually yield a positive culture. But, although many of our patients with definite dissemination have had many gastric washings, the isolation of *C. immitis* from such

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material was rarely successful. In coccidioidal meningitis there are usually several negative spinal fluid cultures before one gets a positive culture. It is tempting to speculate that transition from tissue to culture phase might require more than minimum numbers and minimum viability, or that some "growth factor" or "transforming substance" which could be added to the medium would increase the percentage of positive cultures. At present, however, the diagnosis of coccidioidal infection frequently has to be made without cultural corroboration. Immunologic evidence and the finding of characteristic organisms in the tissue,(3, 8) together or separately, may have to be accepted as reasonable corroboration of the diagnosis. From the broad biologic standpoint it would seem that the *in vivo* reproductive process of the microorganism and the relation between this process and and the host response must be studied primarily in tissue sections(5) even though one might hope to gain additional information from the study of the fungus in tissue cultures or in chemically defined media.

## Methods and Material

This study is based on lesions considered coccidioidal, both originally and on review, from 168 patients studied between 1947 and the present. The basis for the diagnosis, and the distribution and gross description of the lesion, will be discussed in subsequent papers. For careful and brilliant study of material prior to 1950, the writer is deeply indebted to his predecessor, Dr. J. D. Kirshbaum. Forty-three patients provided surgical specimens only; a few of these specimens were from disseminated lesions but most of them were portions of lung resected because of localized disease. The remaining 125 subjects were studied at autopsy. Previous surgical material was available from some of them. Sixty of the 125 were diagnosed as having disseminated coccidioidomycosis, and 65 as having pulmonary coccidioidomycosis. The majority of the lesions in the latter group were incidental findings not directly related to the cause of death.

These figures do not reflect the actual incidence of non-disseminated coccidioidal infection. No attempt has been made to detect all the small peripheral pulmonary granulomas in the autopsy population. Many of the incidental pulmonary granulomas are not amenable to etiologic classification, since they show neither acid-fast bacilli nor fungi. Such lesions have not been included in our figures. Some of these are probably coccidioidal, but there seems to be no way to determine which or how many.

The occurrence of coexistent coccidioidal and tuberculous infection(9) has been of some interest. Tissues from an active tuberculosis service have been used for comparison with tissues infected with *Coccidioides*. The writer has drawn freely upon his previous experience with human and experimental tuberculosis. Tissues infected with *Blastomyces dermatitidis, Histoplasma capsulatum*, and *Cryptococcus neoformans* have been graciously supplied by brother pathologists in exchange for coccidioidal tissue. However, comparative study of coccidioidomycosis and other fungus infections has proceeded only just far enough to show the need for much more.

Tissues have been promptly fixed in formalin and have been embedded in paraffin. Routine sections have been stained with hematoxylin and eosin. Ziehl-Neelsen stains have been used in the search for tuberculous and mixed infections. For the study of *C. immitis* in tissue we have found the Hotchkiss-McManus, or Periodic Acid-Schiff (PAS) stain to be both useful and dramatic. We have used the procedure outlined by Kligman, *et al.*(10)

## Results

No constant or diagnostic features have been discerned among the variegated patterns of human host tissue and cellular response to *C. immitis* infection. These patterns have included caseous pneumonia (fig. 1), fibrinous pleurisy (fig. 2), hard tubercles (figs. 3 to 6), caseous nodules (fig. 7), pulmonary cavities (fig. 8), and small and large masses of polymorphonuclears (figs. 9, 10) both in its range and in the individual patterns the response to *C. immitis* shows an astonishing parallelism to the response to *Mycobacterium tuberculosis*. An intimate mixture of granulomas and micro-abscesses (figs. 11 to 13) is perhaps more suggestive of tuberculosis than of coccidioidomycosis. However such lesions, when actually coccidioidal, have been found to be full of spherules, and it is the spherules which are diagnostic, not the micro-abscess-granuloma mixture.

The most satisfactory basis for a purely histopathologic diagnosis of coccidioidomycosis is the demonstration of a mature sporangium such as that shown in figure 14. In this figure it will be noted that the endospores are approximately the size of polymorphonuclears, that their walls stain heavily with PAS and that there is both diffuse and punctate staining in the zone inside the cell wall. Other

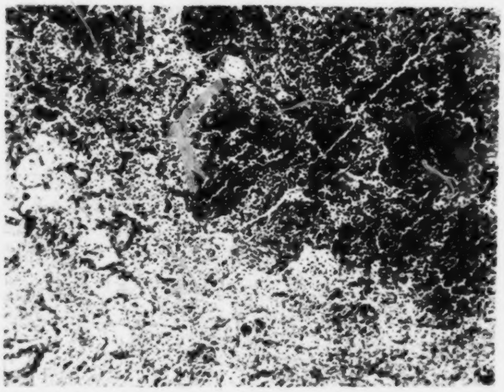

Figure 1. Caseous and organizing pneumonia.

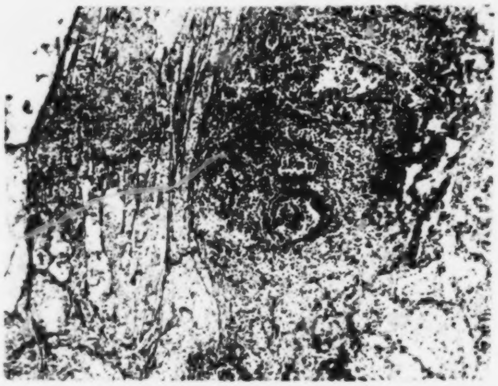

Figure 2. Fibrinous pleurisy.

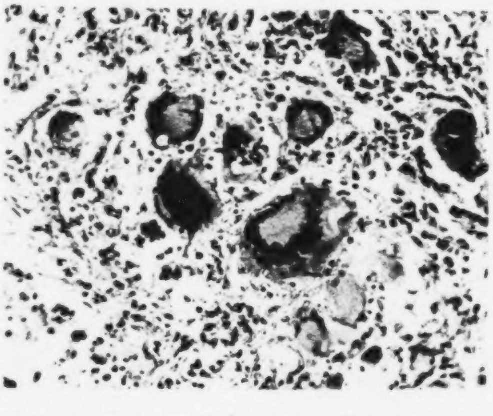

Figure 3. Giant cells in lung, intracellular spherules, and mature sporangium at the right margin.

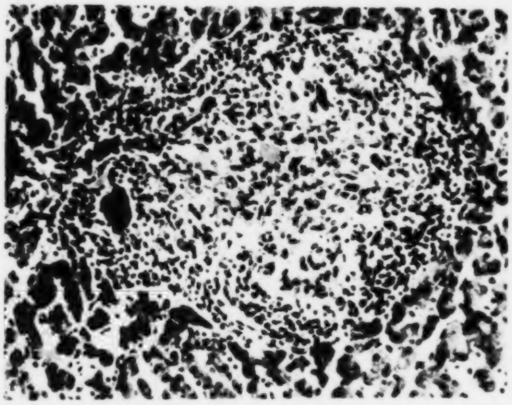

Figure 4. Miliary granuloma in liver.

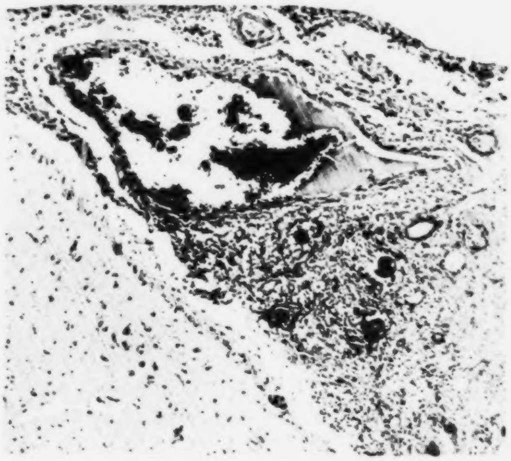

Figure 5. Meningitis — granulomatous reaction.

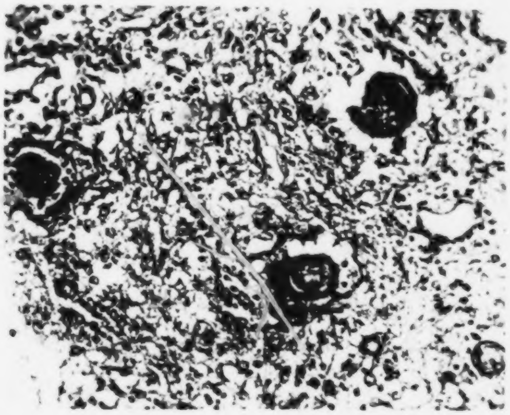

Figure 6. Enlargement of part of field in figure 5, showing mature sporangia.

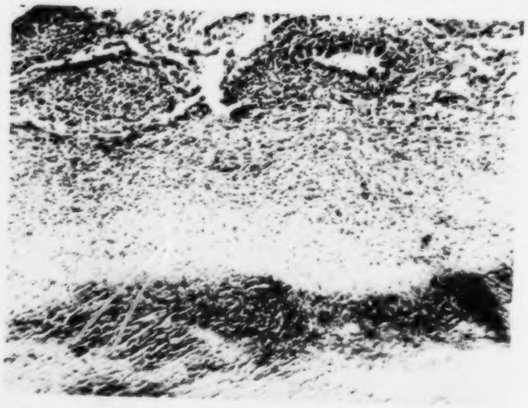

Figure 7. Fibrocaseous nodule.

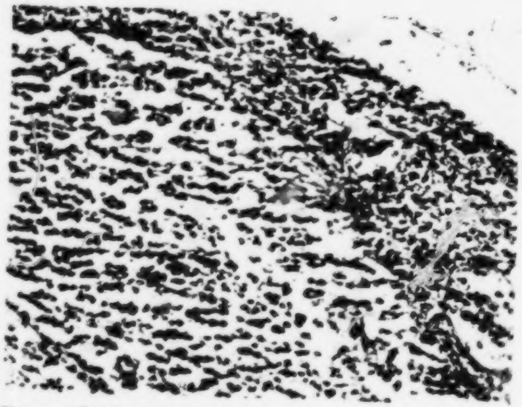

Figure 10. Meningitis-polymorphonuclear reaction.

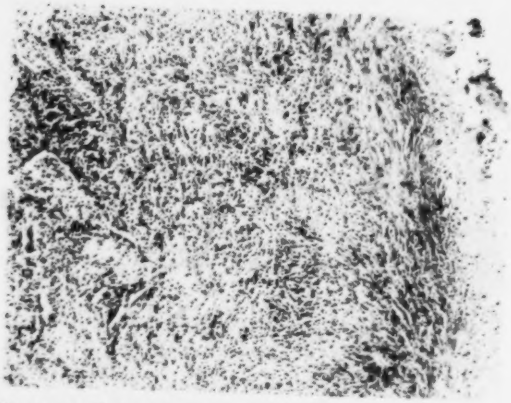

Figure 8. Pulmonary cavity.

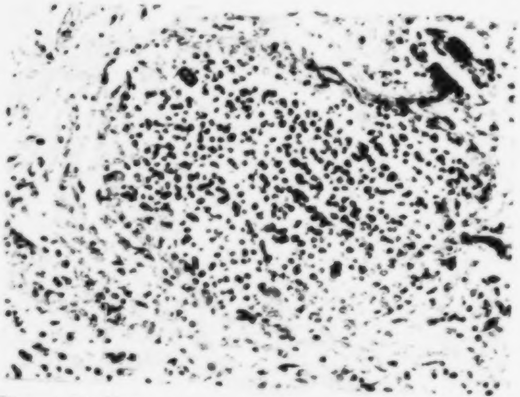

Figure 11. Micro-abscess in small granuloma, lung.

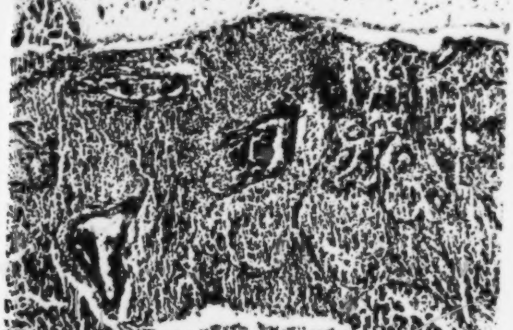

Figure 9. Meningitis-polymorphonuclear reaction.

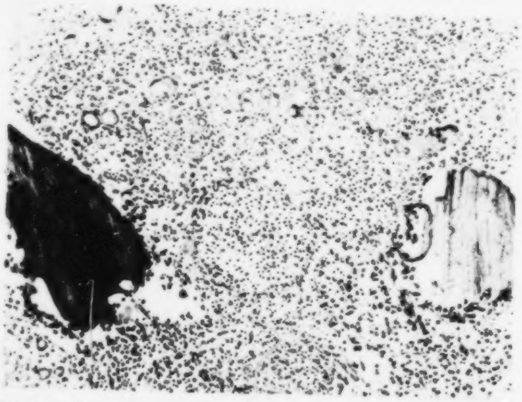

Figure 12. Bone marrow showing micro-abscesses and granulomas.

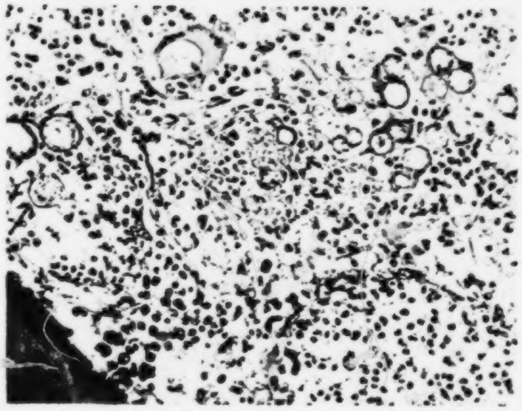

Figure 13. Same, higher magnification. The arrow indicates a "ghost" sporangium containing minute endospores.

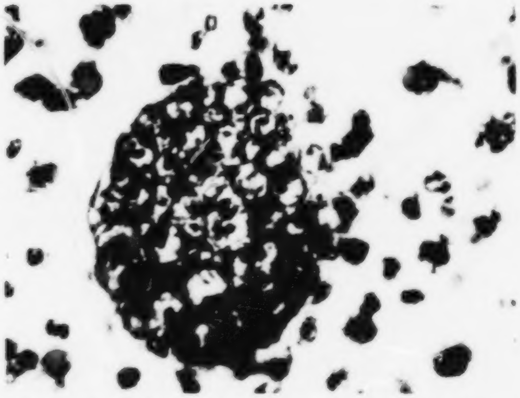

Figure 14. Mature sporangium in pulmonary microabscesses.

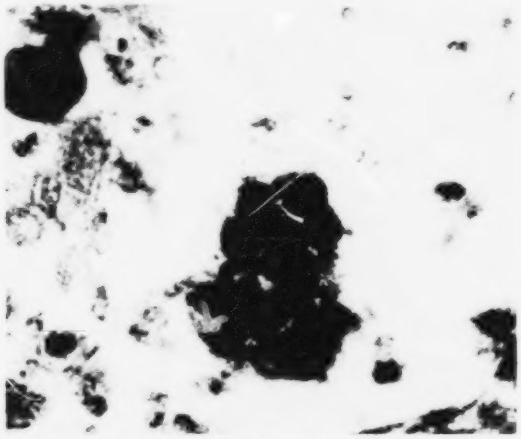

Figure 15. Lung — disintegrating sporangium (center), immature spherule (left). Note tiny vacuolar pattern both in the sporangium and in epithelioid cells.

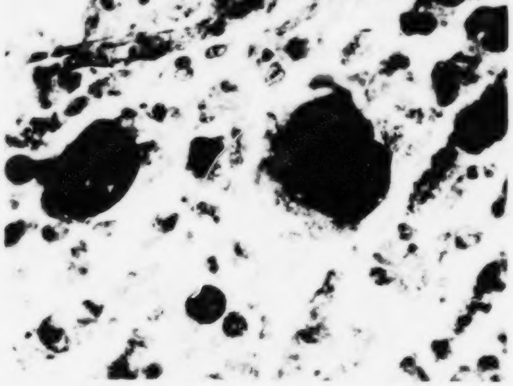

Figure 16. Lung — mature sporangium with adherent tag of giant cell cytoplasm. Several immature spherules at periphery.

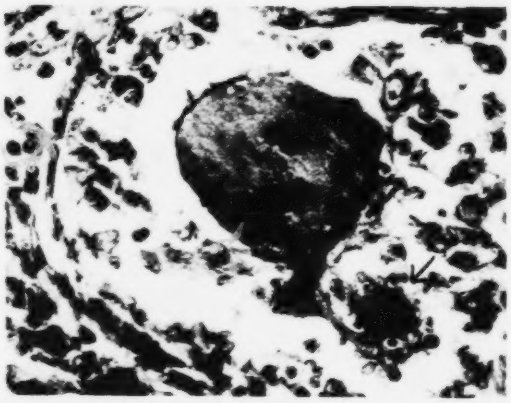

Figure 17. Two giant cells in lung. The one denoted by arrow contains a "ghost" sporangium. The larger giant cell in the center contains Schiff-positive dust, a pair of well-stained spherules, and several tiny structures.

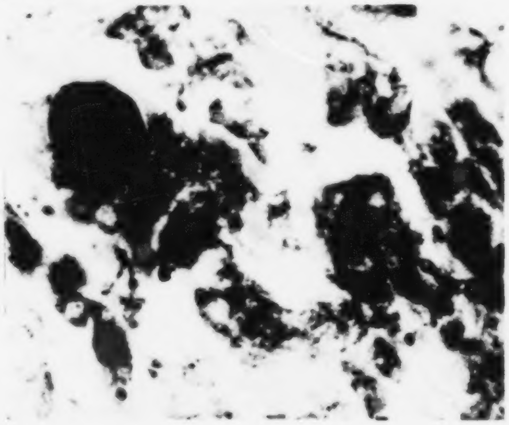

Figure 18. Large immature sporangium, pleura.

mature sporangia are shown in figures 15 and 16. Another sort of structure seems most reasonably interpreted as a "ghost" sporangium – it has thin walls and tiny ring-like or punctate internal bodies (arrows, figs. 13 and 17).

Immature spherules are usually much more common than mature sporangia even in heavily infected tissue. The more characteristic ones range in size from that of endospores in figure 14 to that of the average-sized, mature sporangium (fig. 18). The wall may be the only feature which stains, or there may be both diffuse and spotty staining of the interior (fig. 19). Small shadowy ring-like or punctate structures also occur (figs. 18, 20), and clouds of fine Schiff-positive dust are sometimes conspicuous.

Considerable emphasis has been laid on apparently explosive discharge of endospores from a mature sporangium. However, as Straub and Schwarz have emphasized,(8) disruption of the parent cell need not occur in this dramatic manner. Pairs of immature cells are frequently encountered (figs. 16, 17). A pair of juxtaposed spherules may show either approximate equality or marked difference in size.

The PAS staining may be maintained in apparently nonviable cells, or it may be lost.

Mature sporangia may be found in microabscesses (figs. 14, 15), within giant cells (fig. 6), or with adherent tags of giant cell cytoplasm (fig. 16). Such tags can sometimes be found on serial sections through a sporangium which appeared to be floating free in pus on the first section.

Organisms of all degrees of maturity may be very abundant in an area of pneumonic reaction (fig.21). However in areas of long-standing necrosis or suppuration they tend to be relatively sparse. In sections showing both granulomatous and suppurative areas the spherules are frequently more numerous in the former (figs. 5, 6, 9, 10, 12, 13, 19). By facilitating the distinction between spherules and atypical giant cell nuclei, the PAS stain illustrates the frequent intracellular location of *C. immitis* (figs. 16-20).

In fibrocaseous nodules and in pulmonary cavities, spherules are usually not abundant (figs. 22, 23, 24). Figure 25 shows a clump of spherules in the wall of a cavity. This cavity was in a patient who had a complement-fixation reading of 2+ at a dilution of 1:2 in a postoperative specimen of serum. Her second-strength coccidioidin reaction was positive while her second-strength Purified Protein Derivative was negative. Sputums had shown neither acidfast bacilli nor *C. immitis*. It will be noted that the endospores do not appear to have grown very much since release from the sporangia. Some of both endospores and parent cell walls have lost the ability to react with the PAS stain. Organisms were found only within one small area; most fields in the sections were devoid of them.

Mycelial structures were sometimes encountered in the walls of cavities.(4, 11) One interesting patient – a white man about 21years old – had two cavities resected, both of which showed mycelia. In 1950, he presented himself for treatment, complaining of hemoptysis. X-ray of the chest revealed a large cavity. Numerous sputum specimens cultured on Sabouraud's agar showed *C. immitis* which was confirmed by animal inoculation. A number of sputum cultures on Petragnani agar were negative for tubercle bacilli. Complement fixation on March 2,

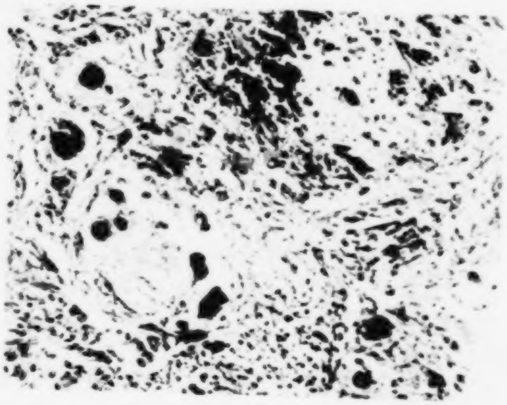

Figure 19. Granulomata and micro-abscess, lung, showing distribution of spherules.

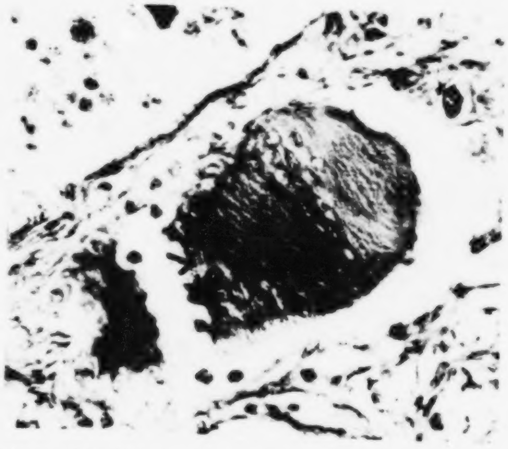

Figure 20. Giant cell in lung containing a small spherule (arrow) and much Schiff-positive dust.

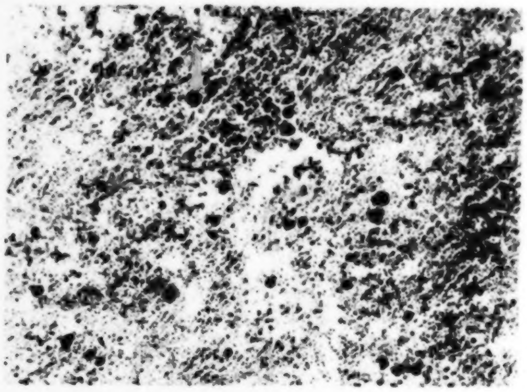

Figure 21. Pneumonic reaction, showing numerous spherules.

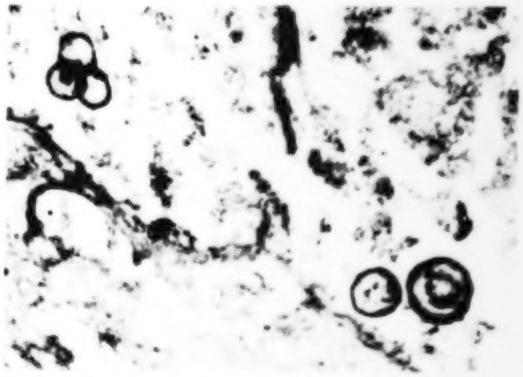

Figure 24. Spherules in caseous area adjoining cavity, lung.

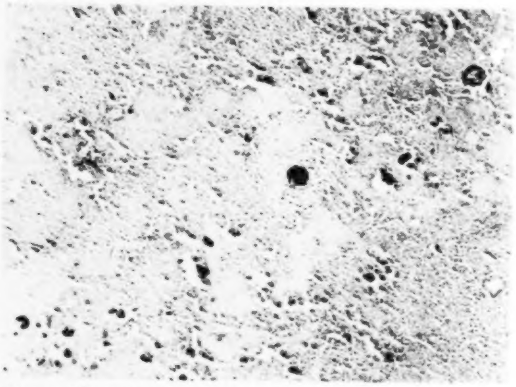

Figure 22. Spherules in caseous nodule, lung.

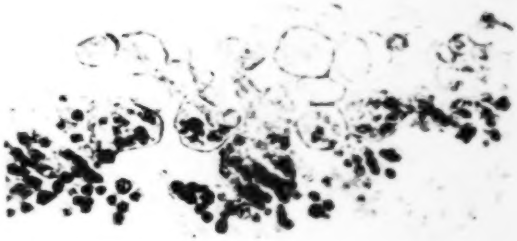

Figure 25. Spherules in wall of cavity.

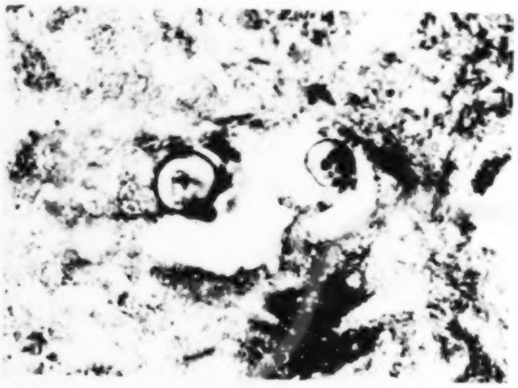

Figure 23. Spherules in caseous nodule, lung.

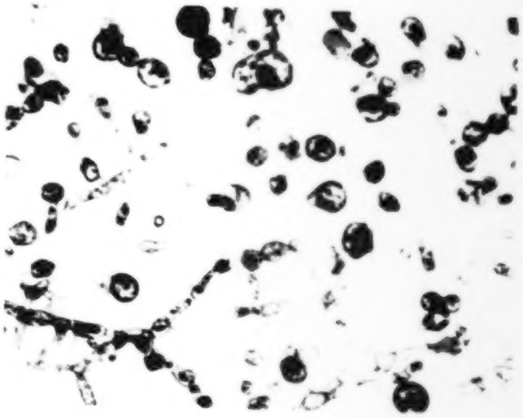

Figure 26. Round bodies and hyphae in wall of cavity.

1951, showed 2+ reaction at a 1:2 dilution. A left upper lobectomy was performed March 15, 1951. Figures 26 and 27 are photomicrographs from sections of the cavity. In 1953, cavitation in the left lower lobe of the lung appeared. Complement fixation was 3+ at a 1:2 dilution and ± at 1:4. Numerous sputum cultures again showed *C. immitis* and no *M. tuberculosis*. Sections of the resected specimen showed typical spherules in caseous material (fig. 28) and a mixture of round bodies and hyphae at the edge of the cavity (fig. 29). In 1956, the patient showed no further pulmonary disease and was doing well.

Another patient, a white man born in 1925, was hospitalized in 194[illegible] for an acute pneumonic process. He returned to the hospital in 1954 with hemoptysis. Coccidioidin skin test was strongly positive, and PPD reaction was negative in both dilutions. Sputum and bronchial aspirate on smear and culture showed neither acid-fast bacilli nor *C. immitis*. *Coccidioides* complement fixation was 2+ at a 1:2 dilution. After surgery it was 3+ at a 1:2 dilution and ± at 1:4. Resection of the right upper lobe of the lung disclosed bronchiectasis and cavitation. Caseous material adjoining the cavity showed round bodies in one area (fig. 30) and a mixture of round bodies and mycelia in another (fig. 31).

For comparison, section of coccidioidal mycelium from Sabouraud's agar is shown in figure 32.

## Discussion

It is convenient to consider the histopathology of coccidioidomycosis in two phases: first, the host tissue and cellular response; and second, the distribution and structure of the microorganisms. A survey of the first phase has revealed nothing very specific but rather a remarkable resemblance to the response to *M. tuberculosis*. It may be important to emphasize that cellular and tissue responses compatible with tuberculosis are not, in the absence of further data, incompatible with coccidioidomycosis. The second phase appears more likely to help us in the diagnosis of coccidioidomycosis, and to analyze its biological peculiarities.

The general features of the reproductive process of *C. immitis in vivo* are well illustrated in sections of heavily infected tissue. Spherules are numerous; indeed the total volume of *Coccidioides* organisms may be astonishing. Mature sporangia are a minority of the spherules, and may be quite hard to find. However, they usually can be found, and often one will find both intact sporangia and sporangia in

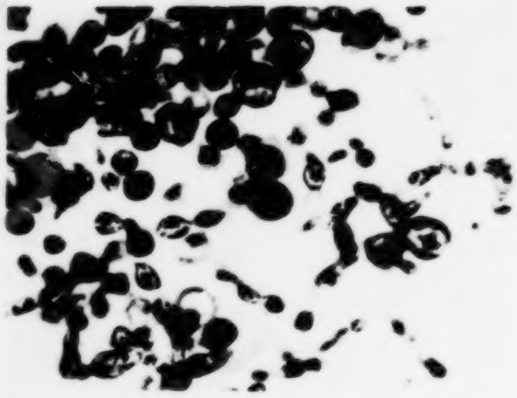

Figure 27. Round bodies and hyphae in wall of cavity.

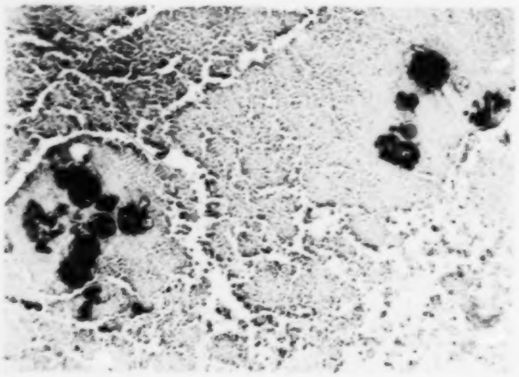

Figure 28. Sporangia in caseous material adjoining cavity.

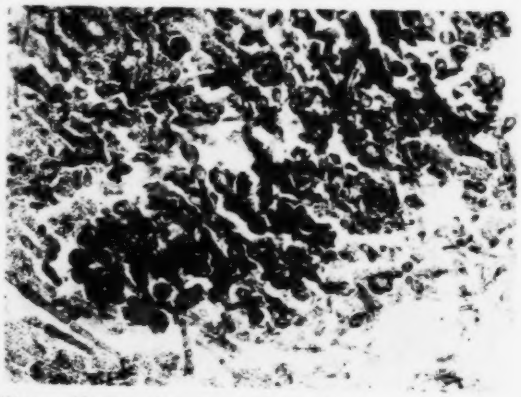

Figure 29. Round bodies and mycelia, wall of cavity, same section as figure 28.

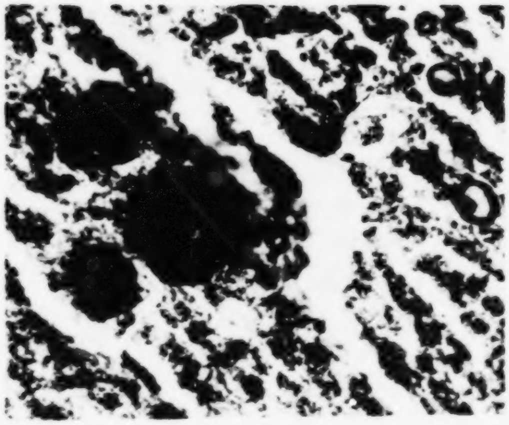

Figure 30. Spherules in caseous material, adjoining cavity.

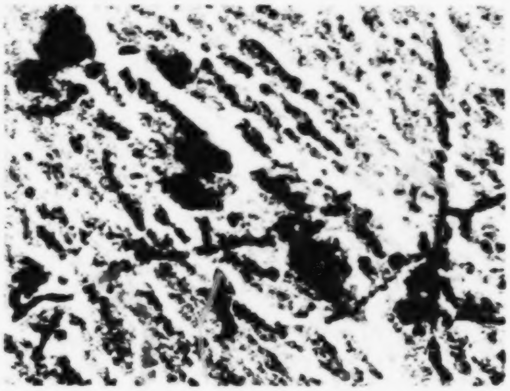

Figure 31. Round bodies and hyphae, same section as figure 30.

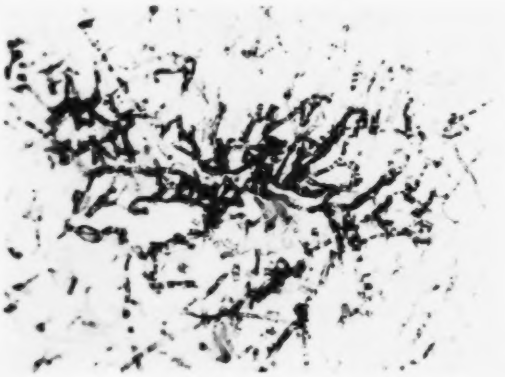

Figure 32. *C. immitis* culture section from Sabouraud's agar fixed and embedded.

various stages of disintegration. The "typical" endospore, like the typical small free spherule, is roughly the size of a polymorphonuclear cell. "Typical " immature spherules range from the size of a polymorphonuclear cell to that of mature sporangia. The frequent groups of two, three, or more spherules may represent merely the surviving or non-scattered progeny of an orthodox sporangium,(8) but it would seem imprudent to discount the possibility of budding or simple splitting.

There is considerable interest in fine Schiff-positive dust and punctate or ring-like structures smaller than "typical" endospores. These are found separately and within structures which appear to be sporangia. While some of these may be unrelated to the fungus, others appear to be either fragmented or degenerated *Coccidioides*.

In view of the amount of work which was originally required to establish the relationship between the *in vivo* spherular phase and the *in vitro* mycelial phase of *C. immitis*,(12) it is not surprising that some workers assumed that *in vivo* growth was never mycelial, and thus the recorded observations of *C. immitis* mycelium in human tissue, particularly pulmonary cavities, were greeted with some reserve. However, we are convinced that *Coccidioides* mycelium does occur in pulmonary cavities and should be looked for.

Mature sporangia, immature spherules, singly, in pairs, and in clumps, small dot or ring bodies, and mycelia, may all occur as manifestations of the reproductive cycle of *C. immitis in vivo*. When several of these are seen together in tissue sections, it is reasonable to surmise there is a relationship. However, in an arrested infection there may be few organisms and those may be in only one phase. The diagnostic value of each of these phases is of some importance. Thus, while a good mature sporangium is for all practical purposes diagnostic, a solitary bit of mycelium in a cavity could hardly be diagnosed as coccidioidal mycelium on tissue section without corroborative cultural or immunologic data. The small dot or ring structures present a number of problems. They may not be viable *C. immitis*, but they occur in *C. immitis* infection and merit further study. It is not inconceivable that under some circumstances they might represent the only formed residue of *C. immitis*, and that they might be confused with *H. capsulatum*. Structures the size of immature *C. immitis* spherules might be confused with the calcospheres seen in tuberculosis. The PAS stain may be helpful here, for in our experience, calcospheres have not been Schiff-positive. Consequently, a caseous lesion containing Schiff-positive round bodies devoid of acid-fast bacilli might reasonably be considered a mycotic lesion rather than a tuberculous lesion. Differentiation in tissue sections between inactive blastomycosis and inactive coccidioidomycosis might be impossible without ancillary epidemiologic and immunologic data.

*C. immitis* appears to be a somewhat inept and unsuccessful organism in human tissue. Its distribution in soil and dust, even in hyperendemic areas appears to be very spotty. When Dr. Ajello and I tried to demonstrate *C. immitis* in air samples, we were impressed with the luxuriant growth of dust organisms which were of no interest in human pathology. Our sampling and cultural procedures were definitely inferior to human lungs as a means of demonstrating circumambient arthrospores. One might be inclined to assume that many arthrospores are necessary for the establishment of a primary pulmonary infection; however, that first transfer from dust to lungs is apparently the only thing at which *Coccidioides* is particularly successful. It does not remain in human tissue very long. Apparently the fungus persists in human tissue only in cases of dissemination and of pulmonary cavitation. Systemic dissemination is very rare in chronic coccidioidal cavitation. In the majority of instances appropriate clinical and immunologic study early in the course of the infection will reveal whether disseminated disease is likely to develop. Dissemination may smolder for months and years, but we have yet to see a convincing example of late dissemination from a pulmonary lesion which has long been local and inactive. If dissemination is to occur, it has to start early. Whether prolonged survival of the organism in long-standing disseminated disease may lead to ultimate survival of the strain in question is a point of some evolutionary, philosophic, and practical interest. It seems possible that in many or most instances invasion of human tissue may represent a dead end for the fungus. The survival of *C. immitis* in fibrocaseous nodules is no better, and perhaps is poorer, than that of *M. tuberculosis*. The prognosis of coccidioidal cavities(7) is far better than was the prognosis of tuberculous cavities in the days prior to modern surgery and chemotherapy. A cavity provided a peculiarly suitable environment for the survival and multiplication of *M. tuberculosis*. Many coccidioidal cavities, on the other hand, fail to show viable *C. immitis*.(11) When viable *C. immitis* is found, it is frequently in the mycelial phase. Usually, *Coccidioides* spherules are not abundant in coccidioidal cavities. This might be compatible with a possible action of inhibiting antibody. It might equally well suggest that suppuration and necrosis *per se* are unfavorable for the survival of *Coccidioides* spherules. The study of tissues in massive disseminated infection gives support for this hypothesis. As Forbus and Besterbreurtje(5) have stated, spherules are often numerous in areas of early necrosis; in our experience, this is seldom true in advanced necrosis. In sections showing both granulomatous and necrotic areas, spherules tend to be more numerous in the former. Despite the very large size of the fungus, it appears to have a decided propensity for establishing itself within epithelioid and giant cells. Many of the intracellular organisms appear hollow or otherwise adversely effected. It is suggested, however, that the necrosis and suppuration brought about by sporangial reproduction may be particularly unfavorable for the survival of *Coccidioides* spherules, and that if the spherular phase is to survive in human tissue for any length of time, it must have a brisk start and must keep moving.

The concept of the precariousness of the foothold of *C. immitis* in the human host, which has been developed largely through clinical and epidemiologic studies, thus appears to be corroborated by histopathologic data. If the idea of the intrinsic ineptitude of *C. immitis* as a human pathogen is correct, the biochemists may be able to confirm it, and to elucidate some of the mechanisms involved.

## Summary and Conclusions

Study of the host response phase of the histopathology of coccidioidomycosis reveals striking parallelism with tuberculosis. Study of *C. immitis* in sections of infected tissue emphasizes variation in grouping, size, structure, and staining properties. The finding of mature sporangia justifies a positive diagnosis of coccidioidomycosis. Structures resembling immature spherules may offer considerable support to the diagnosis of coccidioidomycosis in the absence of mature sporangia and in the presence of ancillary epidemiologic and immunologic data. Some problems of differentiation between coccidioidomycosis and other fungus diseases in tissue sections are briefly considered. The not infrequent occurrence of *C. immitis* mycelium in cavities is confirmed. Histopathologic data suggest that the quick recovery in most cases of localized coccidioidomycosis and the often prolonged course of disseminated coccidioidomycosis need not involve specific immunity but may indicate the intrinsic ineptitude of *C. immitis* as a human pathogen and its poor adaptation to human tissue. It is suggested that though sporangial reproduction tends to produce suppuration and necrosis, these tissue changes are not favorable to survival of *C. immitis* spherules.

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# *Coccidioides immitis*
# Isolation Procedures and Diagnostic Criteria

Libero Ajello, Ph.D.

Undoubtedly *Coccidioides immitis* is one of the most unusual of the fungi that induce human disease. It is a soil fungus (1-5) that is restricted to certain of the semi-arid regions of the New World. In the endemic areas, it causes an essentially benign disease known as coccidioidomycosis in man and lower animals. Untold numbers of rodents and other wild and domestic animals are infected by *C. immitis*, and millions of humans have been, and continue to be, infected by this organism.

*C. immitis* was first isolated and recognized as a fungus by Ophüls and Moffitt (6) of California at the turn of the century. Prior to that time, it was considered to be a protozoan by Posadas (7) and Wernicke (8) of Argentina, the first physicians to study a case of coccidioidomycosis. Rixford and Gilchrist (9) in studying the second and third recorded cases of coccidioidomycosis, which occurred in California, concurred in this belief. However, they were confused by the protean nature of the disease and the tissue forms of the parasite, and, as a consequence, considered that each of their cases was caused by a different species of protozoan. These were placed in the especially created genus *Coccidioides* and named *C. immitis* and *C. pyogenes*. Ophüls (10) called attention to their error in 1905, and *C. immitis* was established as the sole etiologic agent of coccidioidomycosis.

Ophüls (10) described in a very clear and complete manner the morphology of *C. immitis* as manifested in living tissues and on artificial media. He demonstrated that *C. immitis* is a dimorphic fungus. In tissues, it generally develops in the form of unicellular, endosporulating cells (fig. 1a). These cells or sporangia are thick-walled and range in diameter from 20 to 200 microns. Through progressive cleavage the protoplasm of the sporangium is differentiated into a variable number of endospores, which average 2 to 5 microns in diameter. Rupture of the sporangial wall releases these spores, (fig. 1b) and they, when circumstances permit, enlarge, become sporangia, and in turn produce endospores. This cycle is repeated in sequence within the animal body as long as growth conditions are favorable.

When tissues containing sporangia, endospores, or both are inoculated on a variety of media, *C. immitis* develops as a white, cottony mold (fig. 1c). Microscopically, the cottony growth is composed of well-branched. septate mycelium. At maturity, the colony turns brownish, the mycelium becomes closely septate, and the hyphal protoplasm becomes concentrated in alternate cells. These cells develop into arthrospores which typically are biconvex in form with thickened walls and are separated from one another by empty cells (fig. 1d, e). The walls of these adjoining cells are fragile and break readily, releasing the arthrospores which are about 2.5 by 4 microns in size. Characteristically, fragments of the walls of the empty cells remain attached to the arthrospores (fig. 1f). These tags are of diagnostic value as the arthrospores of most other fungi are free of such ornaments.

Arthrospores, when inoculated on suitable media germinate, producing mycelial tubes which elongate, branch, and proliferate to produce new, sporulating colonies. However, when arthrospores are injected into warm-blooded animals, they do not germinate to form mycelium. Instead, they enlarge and become differentiated into endosporulating sporangia. Only under special conditions and with special media can sporangia be induced to form *in vitro*. (11-22)

As a rule, little difficulty is experienced in isolating *C. immitis* from clinical materials. Nutritionally, *C. immitis* is not a fastidious organism (23-24) and grows readily on Sabouraud's dextrose agar, the basic medium of medical mycology. However, the presence of bacteria and saprophytic fungi fre-

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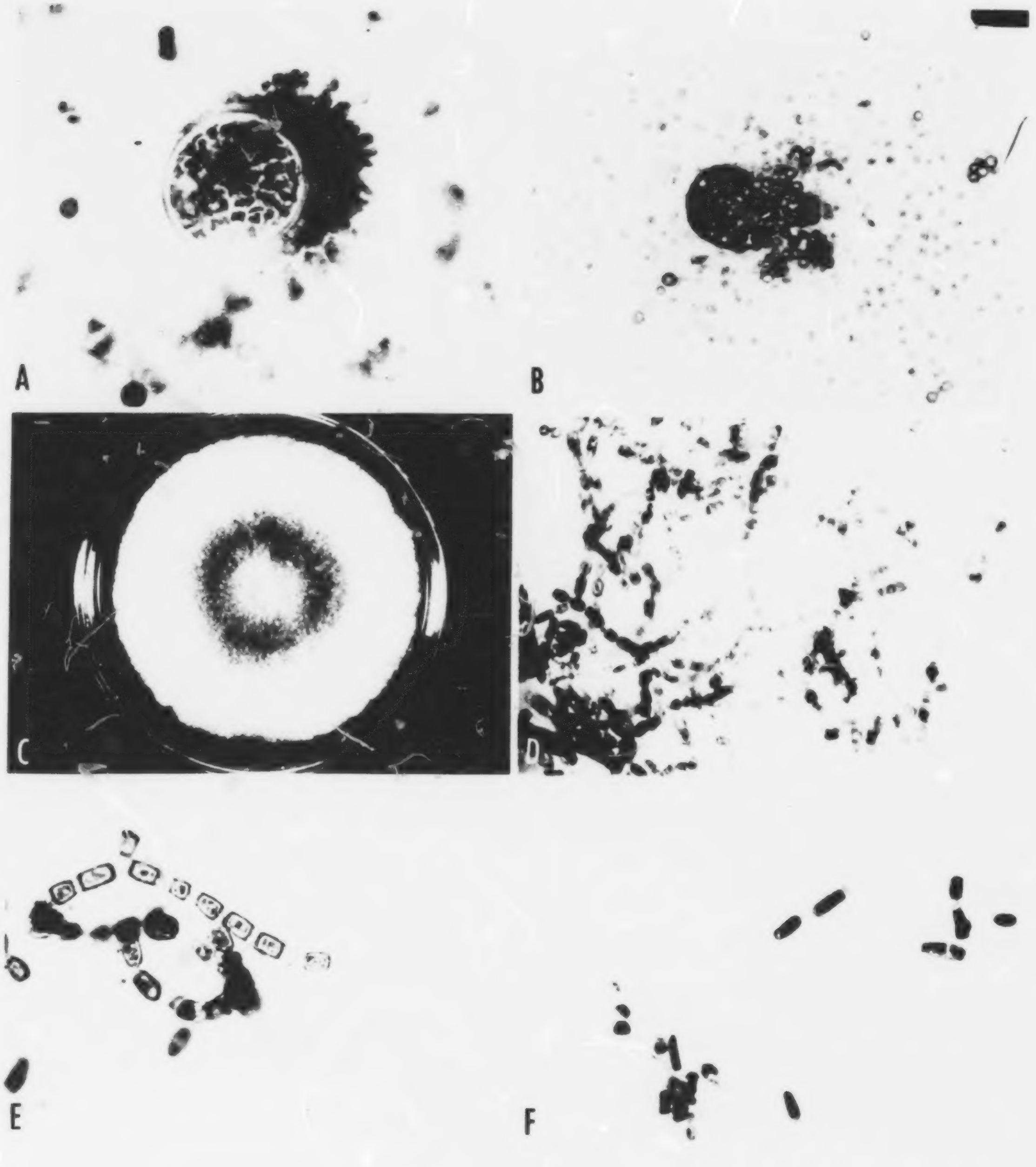

Figure 1 — (A) Mature sporangium of *Coccidioides immitis*. Note the asteroid tissue reaction. Original magnification X970.

(B) Ruptured sporangium with discharged endospores. Gridley stain. Original magnification X480.

(C) Mature colony of *Coccidioides immitis* on Sabouraud dextrose agar.

(D) Mycelium and arthrospore chains. Original magnification X660.

(E) Development of arthrospores in alternate cells of the mycelium. X1320.

(F) Arthrospores with remmants of cell wall attached at their ends. Gram stain. Original magnification X1067.

quently renders its recovery difficult, if not impossible.

With heavily contaminated material, any of several selective isolation media may be used to advantage, or indirect isolation procedures may be employed. A copper sulfate isolation medium virtually specific for *C. immitis* was developed by Smith (25) and is used with great success in many laboratories. Although *C. immitis* grows very slowly and poorly on this medium, very few fungi and bacteria can develop on it. Thus the possibility of isolating *C. immitis* is increased. Littman's agar (26) is also of value in dealing with contaminated inocula. The most recently developed and highly selective medium (27) for pathogenic fungi incorporates anti-bacterial antibiotics and cycloheximide, a remarkable antibiotic that inhibits the growth of most saprophytic fungi but not that of *C. immitis* and most other human pathogenic fungi. A high degree of success is assured in the recovery of *C. immitis* through the use of these differential isolation media.

However, at times even these media may fail to inhibit contaminants. In such circumstances the clinical specimens may be pretreated* with antibiotics or copper sulfate before inoculation either into media tubes or laboratory animals. One thousand units each of penicillin and streptomycin are added to each milliliter of the specimen** which is incubated at 37° C. for at least one hour. An alternate method requires the addition of sufficient copper sulfate to obtain a concentration of 0.04 percent and an incubation period of at least four hours at room temperature.

The antibiotic-treated material is centrifuged at 3200 r.p.m. for 20 minutes. The sediment is resuspended in sterile physiological saline solution and used to inoculate culture media or animals. The sediment from centrifuged copper-sulfate-treated specimens must be washed at least twice with physiological saline to eliminate the copper sulfate which is toxic to mice.

Culture tubes are incubated at room temperature or preferably at 25° C. Screw-capped tubes should be avoided since it has been demonstrated by Hampson (28) that the semi-anaerobic conditions prevailing in sealed tubes tend to inhibit the formation of spores in some strains of *C. immitis* and thereby hinder identification. All cultures should be held for 4 weeks before any are discarded as negative. Growth is slow and at first moist and membranous. Eventually cottony, white, aerial mycelium develops, which tends to become brownish as the colony ages. Synemata-like structures may sometimes be formed by cultures of *C. immitis*. They appear as spicules on the surface of the colonies. However, the structures identified and pictured by Benedek (29) (his fig. 9 a-b) as coremia (synemata) in my opinion are not bundles of mycelium but are the silicified frustules of diatoms which remained connected by their alternate corners.

Microscopic examination of the colony usually reveals the presence of the small, diagnostic, rectangular arthrospores previously described (figs. 4-5). Considerable morphological variation exists among strains of *C. immitis*, as has been emphasized by Friedman and her coworkers. (30) These investigators not only found that a group of 47 cultures formed five different types of colonies, but also that microscopic morphology was highly variable. Although the arthrospores were predominately rectangular to ellipsoidal in form, cantenulate, and separated by adjoining empty cells, others were bizarre in appearance, and several isolates could not be induced to sporulate.

Because of these variations, all cultures believed to be *C. immitis* should be confirmed by mouse or guinea pig inoculation. Mice and male guinea pigs are the animals of choice for use in confirmatory tests and for the indirect demonstration and isolation of *C. immitis* from clinical materials. One ml. quantities of mycelial suspension or pretreated clinical materials are injected intraperitoneally into groups of 2 or preferably 4 mice. These are held for 4 weeks, sacrificed, and their tissues are examined for the presence of spherules containing endospores (figs 2a, b).

Male guinea pigs are injected intratesticularly with 1.0 ml. quantities of the inoculum. *C. immitis* will induce a marked orchitis and even death within two to four weeks time. If death does not ensue, the animals are sacrificed at the end of 4 weeks. Pus from the testes of dead or sacrificed animals is then examined for the presence of spherules.

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* Adapted from procedures used by the California State Department of Public Health's Bacteriology Laboratory, Dr. A. S. Browne, Director.

** Viscous specimens are homogenized by diluting them with physiological saline and shaking with sterile glass beads.

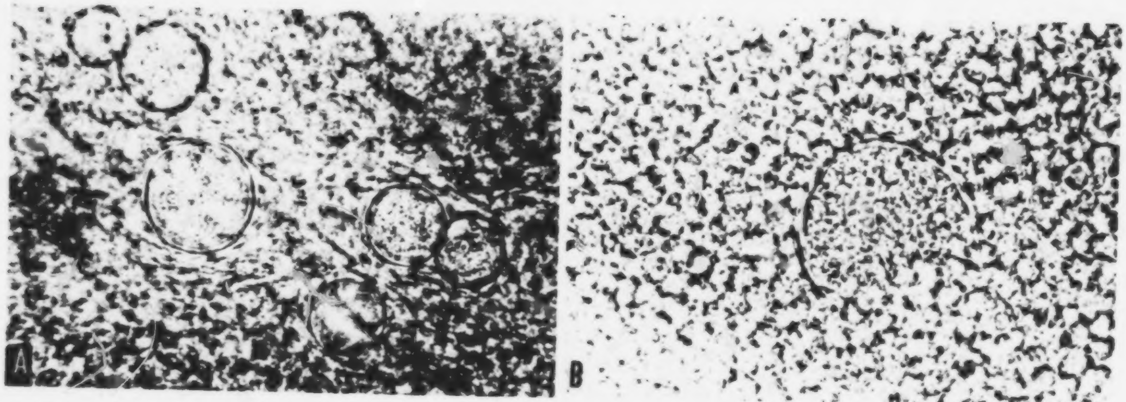

Figure 2 – (A) Lacto-phenol cotton blue mount of exudate from mouse revealing sporangia of *Coccidioides immitis* in various stages of development. Original magnification X220.
(B) Sporangium in fresh mount of mouse tissue. Original magnification X473.

Unfortunately, several investigators have interpreted joined sporangia as representing examples of of reproduction of *C. immitis* by budding.(29,31,32) Smith(33) believes – and it is my opinion as well – that the alleged budding cells represent sporangia that have developed either from joined pairs of arthrospores or endospores.

Frequently arthrospores fail to separate from the mycelium and remain attached to each other by the cell walls of the intervening empty cell. When they are injected into animals, they enlarge and simulate budding – a phenomenon that is well illustrated in the papers of Lubarsky(21) and Baker *et al.*(34) In early stages of enlargement, the tube-like intervening cell is clearly seen connecting the immature sporangia; however, when sporangial enlargement has been completed, the intervening cell is obliterated, and the juxtaposition of the two sporangia deceptively gives the appearance of budding.

In the development of endospores through progressive cleavage of sporangial cytoplasm, endospore initials may fail to be separated. As they enlarge, the liberated but joined endospores, either paired or in larger aggregates, appear to be in various stages of budding.

In addition, it is well to remember that artifacts that resemble budding yeast cells are created by the interaction of fat globules and alkali. Such may be the nature of some of the "budding" cells depicted in DeLamater and Weed's previously cited publication.(32)

Thus, there appears to be no valid basis for postulating that reproduction by budding occurs in the life cycle of *C. immitis*. All the reports of budding by this fungus that are cited and described by Benedek,(29) with the following exception, can be ascribed to errors in interpretation. The budding described by Beaver and Farrer,(35) however, represents true budding, as those investigators were dealing with a case of blastomycosis caused by *Blastomyces dermatitidis* and not one caused by *C. immitis*, as assumed by Benedek.

Since *C. immitis* has been the cause of numerous laboratory infections(36-37) and even a laboratory fatality,(38) extreme care must be exercised in its handling. Unless safety hoods are available, it may be advisable to use only the indirect methods cited previously for the isolation of this fungus. If animal tests cannot be employed, only test tube cultures should be made. Petri dish cultures are too dangerous to handle as the plates break readily, and air currents easily enter them and disperse the numerous, small arthrospores of *C. immitis*. Before manipulation, test tube colonies should be wet down with saline as a further safety measure. This tends to prevent the dispersion of spores from inocula removed from the colony. As a general rule, it is advisable that all white, cottony molds be treated as though they were *C. immitis* until proven differently. Thus, through the use of simple procedures and common sense, the hazards of working with *C. immitis* can be reduced to a minimum.

## Summary

*Coccidioides immitis* is a dimorphic fungus. It exists in living tissues in the form of large endosporulating cells known as sporangia or spherules. In isolation media, *C. immitis* develops as a white, cottony mold. When mature, numerous, small arthrospores are formed from alternate cells of the mycelium. These arthrospores are usually ornamented with fragments of the walls of the adjoining empty cells.

*C. immitis* does not have unusual nutritional requirements, and it is readily isolated and grown on Sabouraud's dextrose agar. However, the presence of bacteria and saprophytic molds in clinical specimens tends to make its recovery difficult if not impossible. With the aid of a variety of selective isolation media and/or pretreatment of specimens with substances that inhibit contaminants success in the isolation of this fungus can be readily achieved.

To avoid the hazards of handling cultures of *C. immitis*, laboratory animals can be used for indirect demonstration of the presence of the organism in clinical specimens.

Animals also may be used to confirm the identity of cultures of *C. immitis*. When introduced into the tissues of laboratory animals, the mycelium and endospores of this fungus are converted into endosporulating sporangia. Animal pathogenicity tests should be made to confirm the identity of all cultures considered to be *C. immitis*. This is almost mandatory because of the wide variations in gross and microscopic morphology exhibited by cultures of *C. immitis*.

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# Question and Answer Period on the Serology of Coccidioidomycosis

Charles E. Smith, M.D., and Charlotte C. Campbell, B.S.

**Question:**

What is the significance of cross-reactions in antigens used in serologic tests for coccidioidomycosis, histoplasmosis, and blastomycosis?

**Answer: (Miss Campbell)**

The problem of cross-reactions in mycotic antigens is one with which we at the Walter Reed Army Institute of Research have been very much concerned ever since we began doing serologic tests approximately 10 years ago. We have discussed it in considerable detail.(1) Although our initial studies were directed to the development of serologic tests for histoplasmosis, we early discovered that we could not afford to limit our activities to this one disease. Most of our patients were military personnel who had resided, travelled, or served tours of duty in areas known to be endemic for coccidioidomycosis and blastomycosis, as well as histoplasmosis. For this reason, we started complement fixation tests simultaneously for all three diseases on each serum. We now have tested over 17,000 sera.

The results obtained in these studies, which include those in collaboration with Smith,(2) leave no doubt that the antigens do cross-react to a marked degree in some sera and not at all in others. This cross-reactivity is associated to some extent with the type, degree, and duration of infection. It is especially strong in the primary pulmonary and primary progressive cases of coccidioidomycosis and histoplasmosis, which all too often are also clinically and roentgenologically indistinguishable. We are less concerned with the marked crossing of *Blastomyces* antigens in the early pneumonitic cases, for there is as yet little evidence that the clinical onset in blastomycosis is ever acute. In fact, the non-specific complement-fixing activity of the *Blastomyces* antigen is sometimes used to good advantage, as it often reacts in sera from the early histoplasmosis and coccidioidomycosis cases before antigens of the homologous agents. On the other hand, cross-reactions decrease and frequently are absent in the cases of coccidioidomycosis, blastomycosis, and histoplasmosis of longer duration. It is wise, nevertheless, to follow the individual case and to refrain from "lumping" general impressions.

Case histories help immeasurably not only in interpreting the tests but also in advancing general knowledge regarding these extremely protean infections.

Coccidioidin, fortunately, is relatively specific in the complement fixation test. Low complement fixation titers of 1:8 or 1:16 are occasionally observed in sera from primary pulmonary histoplasmosis, but they are invariably accompanied by much higher ones of 1:128 to 1:512 with the homologous antigen. As subsequent specimens are tested, even this low cross-reaction drops out. The only danger in this instance is the failure to test with the three antigens simultaneously, since complement fixation titers of 1:8 and 1:16 with coccidioidin are regarded as diagnostic of coccidioidomycosis.

The greater complement fixation specificity of coccidioidin at least gives us a place to start, for the real difficulty is with the other antigens. *Histoplasma* and *Blastomyces* antigens each react to complement fixation titers frequently exceeding 1:32 in sera from early primary coccidioidomycosis, even in those cases in which the complement-fixing antibody to coccidioidin is never developed. The cross-reaction is less difficult to interpret when complement fixation titers are also demonstrable with coccidioidin, for, although the titers with *Histoplasma* and *Blastomyces* antigens may be equal or slightly higher, there have been few instances of this sort in which *Coccidioides immitis* was not the organism recovered from the patient. Similar titers in such cases have ranged from 1:8 to 1:256, indicating that cross-reactions sometimes

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persist in the rapidly disseminating infection of coccidioidomycosis.

This emphasizes the importance of testing serial specimens, both from the standpoint of diagnosis and, in coccidioidomycosis, prognostic evaluation. Cross-reactions may be marked in a specimen taken 2 weeks after onset and much less pronounced or entirely absent in one obtained 2 weeks later. In those cases where they persist at a fairly uniform level, we are at least prompted to intensify our cultural efforts to isolate the specific mycotic agent.

Cross-reactions are even more troublesome in the precipitin and agglutinin tests. In the precipitin test even coccidioidin cross-reacts with sera from primary histoplasmosis and cutaneous blastomycosis. We, therefore, use the collodion agglutination test with histoplasmin almost exclusively in our laboratory, and we interpret the positive reaction as presumptive evidence that an infection is mycotic in origin and not histoplasmosis, *per se*. This does not destroy the usefulness of the test but enhances it. The positive agglutinin reaction rapidly and presumptively differentiates the acute pneumonitic infections due to *Histoplasma capsulatum* or *C. immitis* from similar ones of viral or bacterial etiology, again channeling additional serologic and cultural studies in the right direction. For the tests to provide this presumptive evidence, sera must be tested early and often after onset. Precipitins as well as agglutinins are extremely transitory and in many cases as little as a month after onset are no longer demonstrable.

While on the topic of serologic presumptive evidence in mycotic diseases, it is perhaps pertinent to suggest that clinicians in endemic areas for coccidioidomycosis be increasingly alert for histoplasmosis. Many people who are moving into the Southwest come from areas long recognized as endemic for histoplasmosis, and others come from regions in which the endemicity, although regarded as low, is actually unknown for this disease. To substitute lack of information for proof of absence of histoplasmosis in a particular region is a pitfall from which we in the eastern United States have already suffered. We repeatedly isolate *H. capsulatum* from soils in eastern seaboard States and find primary infections in persons who have never resided in any but supposedly non-endemic areas.(3,4) In view of the cross-reactions, it might be well to consider that it is just as possible for a transient patient with positive coccidioidin precipitins to bring his histoplasmosis with him as to acquire coccidioidomycosis after arrival.

Lest this discourse on the imperfections of serologic tests discourage you from using them, I would remind you of the dominant role that serologic tests have played in changing the clinical concept of coccidioidomycosis and histoplasmosis. Their advantages far outweigh their shortcomings, and it is only the latter that we have discussed. Serologic reactions, even cross-reactions, vary with the individual and the type, degree, and duration of his infection. This circumstance will in some measure still apply when we have more specific antigens.

**Question:**

Is a complement-fixing antibody titer of 1:2 indicative of coccidioidomycosis in a person on whom culture studies have not been carried out?

**Answer: (Miss Campbell)**

It is impossible to interpret a result taken out of context, especially when it involves a titer as low as 1:2. It would first of all be desirable to know whether the serum was simultaneously tested with other fungus antigens and to have a record of the clinical status and residential history of the patient. This low level could mean the beginning of a primary infection that ultimately might be very severe or it could be a residual titer in a patient who had long since recovered from a coccidioidomycosis infection or it could point to a meningeal involvement with poor prognosis. It could also mean that the patient has a moderate to severe primary histoplasmosis, and last. but not least, the reaction could be the result of technical error. In any event, the tests should be repeated on subsequent specimens, and all additional data on the case should be made available. Antibody levels rise and fall, depending on the individual and his particular type of infection. Unfortunately, they do not just arrive at a desired level at a given time for our convenience. We, therefore, cannot urge you too strongly to correlate serologic information with clinical data and other factors in each individual case and to base your conclusions on repeated serologic studies rather than a single specimen.

**Question:**

What is the significance of a positive complement fixation test in spinal fluid?

**Answer: (Dr. Smith)**

Complete fixation of complement by spinal fluid is diagnostic of coccidioidal meningitis. Complement-fixing antibodies pass from maternal circulation into fetal circulation, but they do not pass the blood-brain barrier. In one-quarter of patients with coccidioidal meningitis the cerebrospinal fluid will not fix complement. Therefore, failure to fix complement does not eliminate that diagnosis. Increased cells (especially lymphocytes) and reduced sugar are two findings in spinal fluid which are very important. The first zone ("paretic") colloidal gold curve is frequently seen in coccidioidal meningitis.

**Question:**

Please compare the time relationships of skin sensitivity and diagnostic humoral antibodies.

**Answer: (Dr. Smith)**

The time of the appearance of dermal sensitivity, complement-fixing antibodies, and precipitins has been discussed at length.(5,6,7) In summary, unless there is disseminating disease, dermal sensitivity is established before precipitins develop, and generally precipitins appear before complement is fixed. Precipitins wane first and are relatively infrequent five months after infection is acquired. We recall that precipitins are the only demonstrable, diagnostic humoral antibodies in one-third of those with clinically manifest, primary infections. When complement also is fixed, those antibodies generally persist longer than do precipitins. Indeed, occasionally patients with coccidioidal pulmonary residuals, even cavities with the fungus demonstrable in the sputum, may have persisting complement-fixing antibodies after having lost sensitivity to 1:100 coccidioidin and even to 1:10 coccidioidin. Generally, dermal sensitivity persists longer than do the humoral antibodies, but we are becoming increasingly aware that there are many instances when the relationships are reversed. In this connection it is well to appreciate the importance of a 24-hour reading of the coccidioidin skin test. Especially with long-standing infections, the erythema and induration may have vanished by 48 hours. A good rule is to read at 24 hours and ask the patient who shows no reaction at that time to return the next day if any change develops. Occasionally a reading negative at 24 hours will be positive at 48 hours.

**Question:**

Why do some people with coccidioidal infections in Arizona fail to react to commercial coccidioidin? Is it because the strains of *C. immitis* in Arizona are different?

**Answer: (Dr. Smith)**

There may be various reasons why those with coccidioidal infections may not react. As we have just discussed, the infection may be so long-standing that sensitivity has been lost.

Another reason for failure to react may be that the coccidioidin test was performed before sensitivity was established. Remember that in the first week of clinical illness, one-sixth of patients with primary infections do not react. Retesting subsequently will demonstrate "conversion" of sensitivity and may be diagnostic of primary coccidioidal infection. By the end of the third week of clinical illness, sensitivity will have been established in all those with primary infections unless they are undergoing progressive primary or disseminating disease.

In progressive primary disease, poor dermal sensitivity is associated with ominous prognosis.

Unfortunately, an all-too-common cause is faulty technique in the skin test. During World War II our colleagues at one of the large military general hospitals outside the coccidioidal endemic area claimed that patients rapidly lost their skin sensitivity. From our station hospitals in the San Joaquin Valley, we would transfer to their care patients with unusually long illnesses from primary coccidioidal infections. Within a few weeks after some patients arrived at this general hospital, it was claimed that they failed to react to coccidioidin. One case who allegedly had "reverted" to negative returned to us and when retested again was found to react vigorously to 1:100 coccidioidin. We asked him about his "negative" test. He said that it had not been done by the doctors, and when it was performed, the skin had not shown the little bump it did when we tested him. Evidently, he had not reacted to the subcutaneously administered coccidioidin. Another time we were told that one of the soldiers who had a rather severe coccidioidal primary infection had lost his sensitivity to coccidioidin. Apprehensive that he might be showing the anergy of dissemination, we tested him simultaneously with 1:10 and 1:100 coccidioidin. The next day it was evident that he would have reacted to 1:10,000 coccidioidin, for he had a violent response even to the 1:100 dilution. The stock 1:100 coccidioidin to which he had failed to react had been contaminated and was inert.

When kept refrigerated, 1:100 coccidioidin retains its potency for years. We wondered if failure to react in other cases might be because coccidioidin had deteriorated with age. The Cutter Laboratories provided us with outdated coccidioidin. One lot of 1:100 coccidioidin was 6 years old, and another was 5½ years old. However, both reacted "at par" when tested in known sensitive persons. We should recall the previous comments on the importance of 24- and 48-hour readings, for occasionally a single reading at either time may miss a positive. An interesting phenomenon which is slightly digressive is that sometimes a person who fails to react to 1:100 coccidioidin will give a four plus reaction to 1:10 coccidioidin.

Still another possibility which we occasionally have unearthed is that the patient really does not have a coccidioidal infection. Diagnosis on the basis of a coverslip examination of sputum, spinal fluid, or occasionally even pus is very treacherous. So is identification of *C. immitis* in tissue sections without definite endospores. Equally hazardous is identification of the mycelial form without confirmatory animal inoculation. There is nothing diagnostic by roentgenogram. Sometimes patients, and especially physician-patients, are convinced with no evidence other than their own convictions that they have coccidioidal infections. One must be certain of the criteria of diagnosis before blaming coccidioidin for failure to react.

Thus, there are many possible explanations for coccidioidin failing to evoke reactions in those who claim or even are proved to have *C. immitis* infections. The possibility that strains of the fungus vary in their antigenic capacity was one which we explored and reported upon very early.(5,6) As we discussed, various strains from widely separated geographic areas and from patients with varied clinical manifestations did not differ in their "antigenic coverage." Even coccidioidin made from the same strain which infected the patient evoked the same size reaction as did others. However, as a precautionary measure, multiple strains are used in commercial coccidioidin, and they include three "Arizona strains." This coccidioidin has been the basis for the demonstration of coccidioidal endemic areas as far away as Paraguay and Venezuela. Because some Arizona patients have failed to react to skin tests with coccidioidin, but have demonstrable humoral antibodies, the question has been raised about "antigenic coverage." Yet the same strains are used in the serological antigens as in skin-testing materials. If they "cover" in the former, should they not do so in the latter? Some of us who have struggled with this problem feel that a single strain would do very satisfactorily. My personal hope is that a purified material derived from such a source as the toluene lysate reported on by Dr. Pappagianis will be our optimal skin-testing agent. Certainly, we should explore conscientiously the possibility of strain variations just as we must investigate the known facts of cross-reactions. The problem really is one of technique or incomplete understanding of the status of the infection in the host. Is *C. immitis* of Arizona any different from *C. immitis* of California, Texas, Mexico, Venezuela, Paraguay, or other endemic areas? Possibly, and I shall continue to try to prove or disprove it. Like the now apparently discarded theory of a rodent reservoir and the theory of hazardous human to human transmission, the burden falls on him who claims it. In the meantime, one is derelict in his responsibilities to his patients if he does not explore the possibilities we have reviewed.

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# Preparation and Property of a Complement-fixing Antigen from Mycelia of *Coccidioides immitis*

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Using appropriately prepared "coccidioidins" or culture filtrates of *Coccidioides immitis* as antigens, Smith *et al.* (1) (2) demonstrated the value of skin tests and serological reactions in the diagnosis and prognosis of coccidioidal infections in humans. One of the problems encountered in the course of these studies was the irregularity of appearance of dermal, precipitin, and complement-fixing antigens in these culture filtrates. Customarily, the cultures are incubated for 8 weeks or longer prior to assay of the filtrates. While production of dermal and precipitin antigen has been variable, procuring satisfactory complement-fixing antigen has been especially difficult. A high degree of anticomplementary activity or low complement-fixing titer has necessitated rejection of most filtrates prepared for this serodiagnostic technique.

In a previous report(3) we suggested that autolytic degeneration leads to release of antigens from cells of *C. immitis*, *in vitro* and *in vivo*. If this theory is correct, quantities of antigens might be obtained by inducing lysis of coccidioidal cells rather than waiting several weeks for spontaneous lysis in cultures. In the present report, we describe the production of a highly satisfactory complement-fixing antigen by such an induced lytic process.

## Materials and Methods

The cells of *C. immitis* strain Silveira(4) used in these experiments were grown for 3 to 4 days on a shaker at 34° C. These cultures were produced in 500 ml. Erlenmeyer flasks containing 50 to 75 ml. of 2 percent glucose and 1 percent yeast extract (Difco) broth. Inoculation with 2 to 5 ml. of a young, dense mycelial suspension gave rise to well dispersed mycelial masses which were virtually spore free. These mycelia were harvested by filtration onto two layers of Whatman No. 1 filter paper in a Buchner funnel. The cells were washed several times with distilled water and the wash water removed by suction. This procedure resulted in a coherent sheet of mycelium which could be peeled intact from the filter paper with blunt-tipped forceps. Mycelial mats thus prepared were placed in Erlenmeyer flasks and left in the deep freeze at -12° C. to -15° C. for 2 days. In some instances the cells were not frozen, but treated immediately in the desired manner.

The method of treatment for lysis of the cells was based on a technique used by Drs. E. M. Mrak and H. J. Phaff, Department of Food Technology, University of California, in the preparation of yeast autolysate: A thick suspension of cells was prepared in water and 3 ml. of toluene added for each 100 ml. of water used in making up the slurry. Following the addition of toluene, the suspensions were swirled to disperse the toluene and cells and incubated at 37° C. for 3 days. At the end of this period, the cells appeared somewhat pasty, but could be filtered from the suspension onto filter paper in a Buchner funnel as before.

The lysed cells which remained on the filter were transferred to tared beakers and weighed after drying under reduced pressure at 37° C. Following toluene treatment, or when chloroform or merthiolate was substituted for toluene, the cells were incapable of producing visible growth in glucose yeast extract.

The filtrates (lysates) which were pale yellow to almost amber in color were collected and aqueous 1:1,000 merthiolate added to a final concentration of 1:10,000. The yellow lysates exhibited a fine

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cloudiness which was not removed by slow centrifugation or after standing for several days in the refrigerator. However, centrifugation at 12,000 r.p.m. for 10 minutes clarified the lysates, and sedimented a small amount of brownish material. After initial tests indicated that such clarifying centrifugation did not diminish complement-fixing potency, all subsequent lysates were subjected to to this high-speed centrifugation. Aliquots of the lysates were dialyzed in Visking sausage casing against running tap water for 48 hours to determine the amount of non-dialyzable solutes present. In one instance, 10 ml. of lysate T-2 were dialyzed against three 20-ml. portions of water (with 1:10,000 merthiolate). The contents of the dialyzing bag and the 60 ml. containing dialyzable material were dried at 37° C. under reduced pressure.

Preparations ST-1 and ST-2 were made by toluene treatment of mycelial mats obtained from asparagine medium cultures⁽¹⁾ which had been incubated for 22 days. These were not frozen prior to lysis in toluene water.

The quantitative Kolmer complement fixation test⁽²⁾ was used to assay the lysates with the exception that 0.25 ml. aliquots of serial dilutions of the lysates were added to undiluted, pooled, positive human sera. The latter were obtained from specimens submitted for coccidioidal diagnostic serology and were inactivated for 30 minutes at 56° C. prior to use. Lot 47-63 coccidioidin was used as a standard for comparison of the complement-fixing activity of the lysates. This lot in a 1:8 dilution is currently in use as the antigen in diagnostic tests with human sera.

## Results

The methods of treating various lots of cells as well as the degree of solubilization of cellular components are indicated in table 1. Consistently, about 70 percent of the total dry weight of cells was solubilized by the methods applied. Freezing prior to application of the lytic agent did not influence the results. The cellular components were liberated by exposure to toluene, chloroform, or merthiolate. All three of these agents converted approximately 65 percent of the cell mass into soluble form which could pass through the dialyzing membrane. Approximately 6 percent was retained by the dialyzing membrane. The pH of the lysates was 6.7 to 6.8.

Results of the complement fixation tests in which various lysates were used as antigens are shown in table 2. A most encouraging finding was the complete absence of anticomplementary activity, indicating that the lysates did not bind complement nonspecifically. The standard for comparison, Lot 47-63 coccidioidin, exhibited some anticomplementary activity although well below the 1:8 dilution in which it is used.

In Test 1 of table 2 samples T and T-1 represent aliquots of the same lysate. T-1 was centrifuged at high speed to clarify it while sample T retained the original cloudy appearance. The removal of the fine particles causing cloudiness had no effect on the titer of this lysate.

The effect of chloroform as the lysing solvent is shown in Test 2. Chloroform-treated sample Chl-1 prepared in the same manner as T-3 but with chloroform substituted for toluene appeared to be less active in the complement fixation test in spite of the presence of approximately the same quantity of soluble and nondialyzable solids.

The results of Test 2 (table 2) reflect the low titers obtained with lysates ST-1 and ST-2 from older mixed-strain mycelial mats. Preparation ST-1 gave complete fixation at the 1:4 dilution while ST-2 showed incomplete fixation even at this low dilution. It is apparent from table 1 that toluene treatment of these older surface mycelia liberated much less soluble material than was produced in lysates of the younger cultures. However, the quantity of nondialyzable solids present in the various dilutions used in the serologic tests was similar to that in the other lysates which showed a greater activity in complement fixation.

Test 3 was carried out to determine whether freezing the mycelium before treatment with toluene increased the yield of complement-fixing antigen. Sample T-6 which had not been prefrozen appeared to have as high if not slightly higher activity than its prefrozen counterpart T-5.

In order to determine if the antigenic activity resided in that portion of the lysate which did not pass through a dialyzing membrane, a sample T-2 was dialyzed and compared with whole T-2 lysate (table 3). In addition, a sample of T-2 which had been dialyzed and then dried, was reconstituted to to the original volume with the dialyzable fraction which had been dried in a similar manner. The comparative results of whole T-2, dialyzed material DT-2, and the dried and reconstituted DT-2 (reconst.) are presented in Test 4. Dialysis apparently removed little if any of the complement-fixing capacity of T-2. However, that sample which had been dried and reconstituted after dialysis showed

## TABLE 1

## PREPARATION AND YIELD OF LYSATES FROM *COCCIDIOIDES IMMITIS*

| Lysate, Strain, Treatment | Total Dry Cell Wt. | Non-soluble Cell Residue | | Soluble Solids Freed by Toluene, etc. | | | Soluble Solids | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Non-dialyzable | | | Dialyzable | |
| | g. | g. | % | g. | %* | mg/ml** | g. | %* | mg/ml** | g. | %* |
| T-1 Silveira, frozen, toluene | 4.15 | 0.90 | 21.7 | 3.25 | 78.3 | 26 | xxx | xxx | xxx | xxx | xxx |
| T-2 " " " | 4.64 | 1.34 | 28.9 | 3.30 | 71.2 | 22 | 0.30 | 6.5 | 2 | 3.0 | 64.7 |
| T-3 " " " | 3.70 | 1.10 | 29.7 | 2.60 | 70.3 | 17 | 0.22 | 5.97 | 1.5 | 2.38 | 64.3 |
| Chl-1 " " chloroform | 3.78 | 1.03 | 27.2 | 2.75 | 72.7 | 15 | 0.23 | 6.06 | 1.3 | 2.52 | 66.6 |
| T-4 " not frozen, toluene | 2.21 | 0.60 | 27.1 | 1.61 | 72.8 | 6.5 | xxx | xxx | xxx | xxx | xxx |
| M-1 " " " merthiolate | 2.07 | 0.62 | 29.9 | 1.45 | 70.0 | 5.2 | xxx | xxx | xxx | xxx | xxx |
| T-5 " frozen, toluene | 3.84 | 1.20 | 31.2 | 2.64 | 68.8 | 12 | 0.16 | 4.09 | 0.7 | 2.48 | 64.7 |
| T-6 " not frozen " | 3.99 | 1.11 | 27.8 | 2.88 | 72.2 | 12 | 0.20 | 5.07 | 0.85 | 2.68 | 67.1 |
| ST-1 mixed,[+] not frozen, toluene | 14.39 | 8.93 | 62.1 | 5.46 | 38.0 | 14 | 0.51 | 3.52 | 1.3 | 4.95 | 34.5 |
| ST-2 "[+] " " " | 8.67 | 4.82 | 55.5 | 3.85 | 44.3 | 11 | 0.35 | 4.04 | 1.0 | 3.50 | 40.3 |

* Percent based on total dry cell weight.

** Mg. per ml. of final lysate.

[+] Mixed strains of *C. immitis* as used by Smith (1948) in preparation of coccidioidin.

xxx Not done.

## TABLE 2

## ACTIVITY OF DIFFERENT LYSATE PREPARATIONS OF *COCCIDIOIDES* MYCELIA IN THE COMPLEMENT FIXATION REACTION

| Test | Antigen | Anticomplementary Titration (Reciprocal of Antigen Dilution) | | | | | Antigenic Titration (Reciprocal of Antigen Dilution) | | | | | | | Hemolytic Control** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Un | 2 | 4 | 6 | 8 | 4 | 8 | 12 | 16 | 24 | 32 | 48 | |
| 1 | Lot 47-63* | 4 | ± | 0 | 0 | 0 | 4 | 4 | xxx | 4 | 3 | 2 | 1 | 4 |
| | T | 0 | 0 | 0 | 0 | 0 | 4 | 4 | xxx | 4 | 4 | 4 | 4± | 4 |
| | T-1 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | xxx | 4 | 4 | 4 | 4± | 4 |
| | T-2 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | xxx | 4 | 4 | 4 | 3 | 4 |
| 2 | Lot 47-63 | 4 | 4 | ± | 0 | 0 | 4 | 4 | xxx | 4 | 4 | 4 | 3 | 4 |
| | T-3 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | xxx | 4 | 4 | 3 | 1 | 4 |
| | Chl-1 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | xxx | 2 | 1 | ± | ± | 4 |
| | St-1 | 0 | 0 | 0 | 0 | 0 | 4 | 2 | xxx | ± | 0 | 0 | 0 | 4 |
| | St-2 | 0 | 0 | 0 | 0 | 0 | 3 | ± | xxx | 0 | 0 | 0 | 0 | 4 |
| 3 | T-2 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 4 | 4± | 2 | 2 | 1 | 4 |
| | T-5 | 0 | 0 | 0 | 0 | 0 | 4 | 4± | 2 | 2 | 1 | ± | 0 | 4 |
| | T-6 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 4± | 2 | 2 | 1 | 0 | 4 |

* Lot 47-63 was the control antigen used by Dr. C. E. Smith in diagnostic coccidioidal serology.

** Hemolytic control; *Coccidioides* antigen, saline plus sheep cells.

± to 4 Slight to no hemolysis; fixation of complement by *Coccidioides* antigen.

0 Complete hemolysis; no fixation by *Coccidioides* antigen.

xxx Not done.

## TABLE 3

ACTIVITY OF *COCCIDIOIDES IMMITIS* LYSATES AFTER (1) DIALYSIS AND (2) DIALYSIS AND RECONSTITUTION IN THE COMPLEMENT FIXATION REACTION

| Test | Antigen | Anticomplementary Titration (Reciprocal of antigen dilution) | | | | | Antigenic Titration (Reciprocal of antigen dilution) | | | | | | | Hemolytic Control* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Un | 2 | 4 | 6 | 8 | 4 | 8 | 12 | 16 | 24 | 32 | 48 | |
| 4 | T-2<br>Whole lysate | xxx | xxx | xxx | xxx | xxx | 4<br>(1.3) | 4<br>(0.66) | xxx | 4±<br>(0.33) | 2<br>(0.2) | 2<br>(0.16) | 0<br>(0.11)** | 4 |
| | DT-2<br>Dialyzed, lysate | 0 | 0 | 0 | 0 | 0 | 4<br>(0.1) | 4<br>(0.05) | xxx | 3<br>(0.025) | 2<br>(0.017) | 1<br>(0.015) | 0<br>(0.008) | 4 |
| | DT-2<br>Dialyzed, reconstituted | 0 | 0 | 0 | 0 | 0 | 4±<br>(1.3) | 2<br>(0.066) | xxx | ±<br>(0.33) | ±<br>(0.2) | 0<br>(0.16) | 0<br>(0.11) | 4 |

* Hemolytic control; *Coccidioides* antigen, saline plus sheep cells.

** Numbers in parenthesis give the amount of soluble solids present in the 0.25 ml. of antigen solution used in each tube

xxx Not done.

a considerable drop in activity. The parenthetical figures shown in Test 4 represent the milligrams of soluble material present in 0.25 ml. aliquots of the various dilutions used in the test. Thus, only about one-tenth of the soluble solids liberated by toluene lysis, that is, the nondialyzable portion, was active in complement fixation.

The activity of the other lysates in complement fixation was also calculated on a dry weight basis and has been plotted in figure 1. Complete fixation of 2 units of complement appears to have been accomplished in the range of 0.2 to 0.5 mg. of soluble solids. This would represent about 0.02 to 0.05 mg. of nondialyzable complement-fixing fraction, and indicates a rough stoichiometric relationship. The lesser activity of lysates from older mycelium is again apparent from the data of figure 1.

Close to 50 percent of the nondialyzable complement-fixing fraction of the various lysates consisted of carbohydrate (anthrone reactive material expressed as glucose). Paper chromatography of acid hydrolyzed nondialyzable T-2 revealed the presence of several ninhydrin reactive spots appearing to represent amino acids, as well as reducing sugars. It would appear, therefore, that we are again dealing with a nitrogenous polysaccharide complex as was the case with the precipitin antigen.(3,5) The results of a precipitin test using lysates T-1 and T-3 are shown in table 4. While clearly positive precipitin reactions were obtained with two dilutions of T-1, other dilutions of this lysate and of T-3 were of a questionable type.

A thorough study of the stability of the lysates as antigens has not been undertaken. However, lysates T-1 and T-2 have shown little if any change in complement-fixing activity after 6 weeks at 4° C. Furthermore, lyophilized samples of T-1 and T-2 showed a similar stability even after 3 months at room temperature.

## Discussion

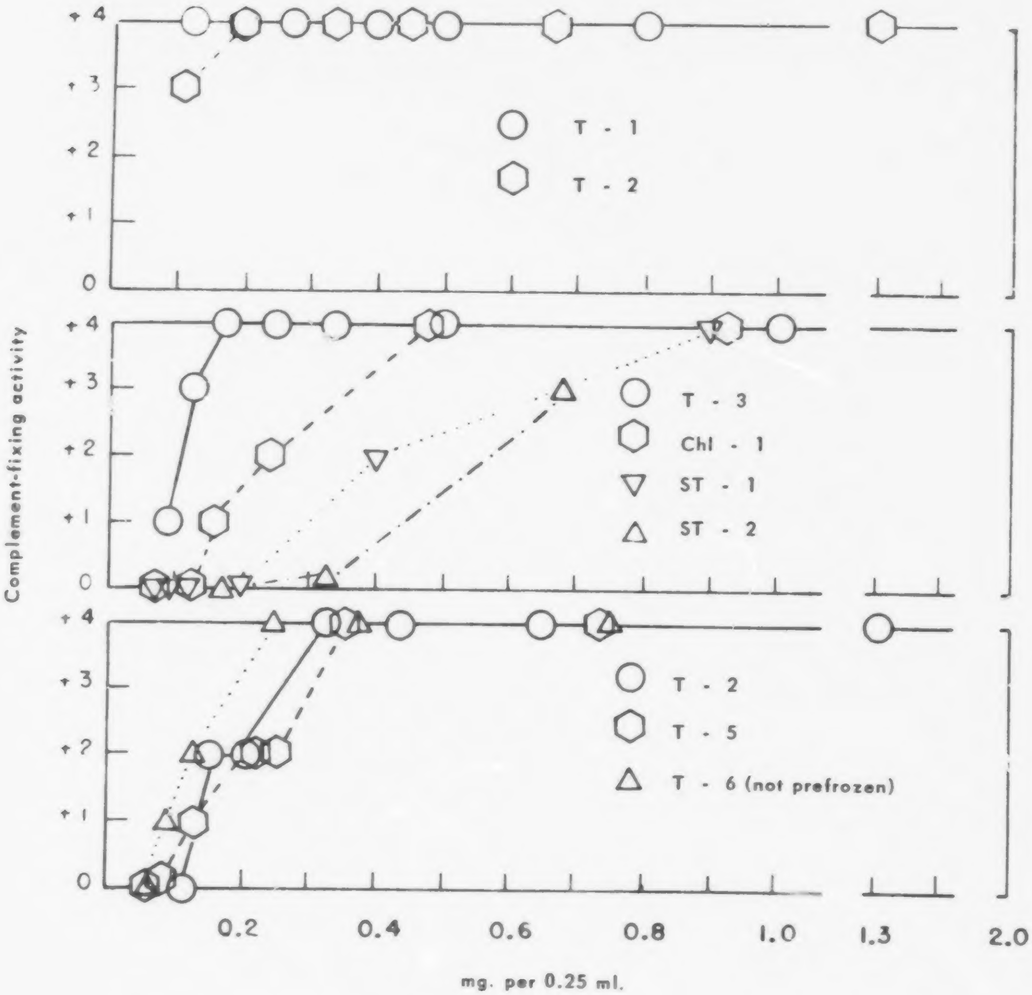
The postulated release of antigens by autolysis of *C. immitis* is supported by the present experiments. Initially, we conjectured that the polysaccharide containing precipitin and dermal antigens of coccidioidin would survive lysis better than the apparently more labile complement-fixing antigen. However, under conditions of induced lysis, especially with toluene, and in the absence of anticomplementary properties, the complement-fixing antigen has been recovered.

When the active, nondialyzable portion of lysate

T-2 was found to contain 50 percent carbohydrate, we were interested to know if this would serve as an antigen for precipitin reactions. The results thus far obtained do not permit a definite answer. The possibility exists that a single antigen might induce the formation of, and react with, complement-fixing and precipitin antibodies as discussed by Kabat.(6) However, the findings of Smith(2) and Hassid *et al.*(5) indicated that different antigens were responsible for complement fixation and dermal or precipitin reactions. The latter activities appear to be heat stable while the former is destroyed by heat. Furthermore, we have recently confirmed the findings of Hassid *et al.*(5) that the alcohol-precipitated, nitrogen-containing polysaccharide which is active in precipitin and skin tests did not fix complement in the presence of antibody. This substance was not anticomplementary. Seibert *et al.*(7) have recently shown that tuberculopolysaccharide, which also is not anticomplementary, may nonetheless interfere with complement fixation by immunogenic antigen (BCG). It would be important

FIGURE 1

COMPARATIVE ACTIVITY OF LYSATES OF *COCCIDIOIDES IMMITIS* IN THE COMPLEMENT FIXATION TEST BASED ON DRY WEIGHTS OF SOLUBLE SOLIDS PRESENT



## TABLE 4

### ACTIVITY OF TOLUENE LYSATES OF *COCCIDIOIDES IMMITIS* IN THE PRECIPITIN TEST

| Antigen Dilution | Positive Coccidioidin | Lysate T-1 | Lysate T-3 |
|---|---|---|---|
| undil. | ++++ | –* | –* |
| 1:10 | ++++ | | |
| 1:20 | | ++++ | –* |
| 1:40 | ++++ | ++++ | –* |
| 1:80 | | –* | –* |
| 1:160 | | –* | –* |

*Small flocs present but not typical coherent "button" of precipitate.

to know if coccidioidin polysaccharide has a similar action and if it has a disease-promoting activity proposed by Seibert for the tuberculopolysaccharide.

As yet, we cannot be certain that there is more than one antigen involved in the above-mentioned serological and skin tests. It is possible that autoclaving or ethanol precipitation of coccidioidin polysaccharide destroys molecular integrity which is essential in complement fixation. In the course of lysis by toluene treatment or fortuitously in some old cultures, the complement-fixing component is preserved. It would seem possible, from the retention of complement-fixing ability in toluene lysates, that immunogenicity may also be a property of these soluble cellular components.

The ease with which toluene lysis can be carried out may provide a method for producing antigens from other organisms, for example, *Histoplasma capsulatum* and *Blastomyces dermatitidis*. Cross-reactions between fungus antigens and heterologous sera are known to occur in the systemic mycoses.(2,3) It remains to be seen if toluene or similarly induced lysis can provide antigens with minimal cross-reactivity.

## Summary

In an effort to obtain cell-free antigens, mycelia of *C. immitis* were suspended in water and lysed by adding toluene and incubating at 37° for 3 days. The resultant lysates were found to be active as complement-fixing antigens in relatively high dilution when tested with sera from proved, human cases of coccidioidomycosis. These lysates were devoid of anticomplementary activity. The complement-fixing activity was retained in the nondialyzable fraction of the lysate, which contained about 50 percent polysaccharide (anthrone reactive material) and on hydrolysis showed the presence of ninhydrin reactive substances (amino acids) and reducing sugars.

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# The Veterans Administration-Armed Forces Cooperative Study on Coccidioidomycosis

Milton Huppert, Ph.D.

The foundation for a group study of coccidioidomycosis was laid in the summer of 1956, during preliminary meetings convened with the encouragement of the Veterans Administration Central Office, and attended by individuals familiar with the clinical and laboratory problems involved. Two major points were made at this time: (1) that real problems existed which could be solved only in a concerted effort by an organized group of investigators; (2) that a report form should be prepared for the collection of available information. The report form would serve as the beginning of a case registry and as the source of material for the establishment of future protocols. Committees were appointed to prepare the way for a formal meeting of a study group.

The Veterans Administration-Armed Forces Coccidioidomycosis Cooperative Study was established by a Committee on Plans and by a Laboratory Committee, which met in Los Angeles in November 1956. These meetings were attended by representatives from hospitals and laboratories in the San Francisco area, from the Veterans Administration Central office, from the Veterans Administration hospitals, and from the three branches of the Armed Forces. Many aspects of the problem were discussed, some briefly and some in detail. (1) Plans were made to issue a bulletin containing tentative clinical and laboratory standards in addition to the report form. This paper will review only the following topics: the report form, the laboratory diagnostic standards, the safety precautions for personnel working with cultures of *Coccidioides immitis*, and the culture bank.

## The Report Form

The completed report forms will contain information about the epidemiology of coccidioidomycosis in relation to race, occupation, and presence in endemic areas; the symptoms, signs, and roentgenographic findings; the course of the disease; the results and complications of therapy. The laboratory data will include mycology and serology, pathologic studies of resected pulmonary lesions, and autopsy findings. The immediate objectives are: (1) to determine the number and types of cases available for future studies; (2) to test the mechanics of the cooperative study; (3) to serve as the first step in establishing a case registry. The retrospective data to be collected by the report form were limited to all veterans and Armed Forces personnel admitted to hospital between January 1, 1955 and January 1, 1957. The dependents of these personnel were excluded because of the difficulty in obtaining satisfactory follow-up. It was anticipated that the collection of these data would eventually include cases prior to the dates chosen, but that this would wait upon the first limited trial of the mechanics and content of the form.

## The Laboratory Diagnostic Standards

The topics discussed in recommending the minimum standards for the laboratory diagnosis of coccidioidomycosis included the collection of the specimen, culturing the specimen, the handling and identification of the culture, the demonstration of pathogenicity for experimental animals, and the patient's serology. It was recognized that the only absolute criterion in the diagnosis of coccidioidomycosis is the isolation and identification of *C. immitis*, but that it is not always possible to accomplish this. In such instances certain other criteria are acceptable as diagnostic. These include the demonstration of characteristic, mature, endosporulating spherules in histopathological material, and, in association with active disease, the conversion from negative to positive of the skin, precipitin, and complement-fixation tests. Increasing serological titers in the complement fixation test are also diagnostic.

The following are some of the more pertinent recommendations.

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In pulmonary disease a series should be run consisting of a 24-hour sputum specimen on each of three successive days. If no sputum is raised, then gastric contents or secretions from the tracheo-bronchial tree should be tried. In cases of meningitis, the entire spinal fluid specimen should be passed through membrane filters and the filters cultured.(2)

A minimum amount of time should be spent in the microscopic examination of wet mounts of fresh specimens. This technique is frequently fruitless and, at best, provides only presumptive evidence which must be confirmed by culture, animal inoculation, or serology.

Each specimen should be cultured on at least two media; Sabouraud's glucose agar, and Sabouraud's glucose agar with antibiotics.(3)

For positive identification of a culture,one cannot rely exclusively on gross colonial characteristics or microscopic morphology. A viable suspension should be inoculated into experimental animals. When these animals are autopsied or sacrificed, endosporulating spherules should be demonstrated.

The techniques of C. E. Smith and his collaborators(4) were recommended for the demonstration of complement-fixing antibodies and precipitins. A minimum of two specimens separated by a one-month interval should be taken. At least 5 ml. of serum must be available in the specimen.

Part of the tissue obtained at either surgery or autopsy should be homogenized. The homogenate should be cultured and also injected into experimental animals.

## The Safety Precautions

Material which may yield cultures of *C. immitis* should be inoculated on media in either test tubes or bottles, never in Petri dishes. All manipulations of cultures of this fungus should be done in a bacteriological hood. Where such equipment is not available, tubes or bottles with caps containing a rubber-diaphragm should be used. Suspensions for inoculating animals can be prepared by injecting suitable fluid through the rubber-diaphragm, shaking the culture, and withdrawing the suspension. With this technique there is no need to open a viable culture of *C. immitis.*(5)

Personnel working with cultures should wear face masks at all times. Additional protective clothing is advisable. Surgical gloves should be worn when handling infected animals. All coccidioidin-negative laboratory personnel who handle *C. immitis* cultures should be skin-tested every 3 months and X-rayed every 6 months.

## The Culture Bank

The committee members agreed that it would be most desirable to establish a *C. immitis* culture bank as a source of reference cultures for use in future investigations. The facilities of the Mycology Research Laboratory, San Fernando Veterans Administration Hospital, were designated for this purpose.

The culture bank has been established and is in operation. Present planning involves an intensive investigation of old stock and newly acquired strains. An attempt will be made to characterize each culture according to the following properties: cultural and physiological types, pathogenicity for experimental animals, sensitivity spectrum to chemotherapeutic agents as they become available, and immunological types if such a differentiation is possible.

Cultures should be shipped to the bank by registered mail to insure careful handling in transit. The cultures may be prepared for shipment in either of two ways: (1) grown cultures should be covered with sterile mineral oil; (2) cultures may be transferred to fresh media and shipped immediately, before growth has occurred. All cultures should be grown in heavy-walled, screw-cap bottles or tubes and packed in cotton in a double mailing carton.

## Conclusion

There are many questions which have not been answered satisfactorily in the 20 years during which the clinical manifestations of the disease have been recognized. The study group will seek answers to some of these.

What is the ultimate fate of cavitary and non-cavitary coccidioidal lesions of various sizes?

How many asymptomatic cavities later become symptomatic?

When is surgery indicated, and for what type of lesion? When is surgery contraindicated? What type of pulmonary resection is preferable in removing cavities? For instance, is lobectomy preferable to segmental resection?

What is the fate of patients who had acute coccidioidal disease without apparent residual lesions?

What will be the significance of serological tests on patients undergoing surgery or chemotherapy?

Are there different biologic and/or immunological types of *C. immitis?* Are there avirulent strains of this fungus?

What is the significance of hyphae in a cavity? What is the danger of contagion? Is there any indication for immunization? If so, can an effective vaccine be developed?

The importance of this disease will undoubtedly increase. In addition to the thousands of Armed Forces personnel exposed each year, the population of the States in the endemic areas is increasing by at least one-half million persons each year. It may be anticipated that as many as 90 percent of those moving to endemic areas will develop positive skin tests within 10 years. This will present a problem of considerable magnitude for the hospitals and laboratories not only in the endemic areas but also throughout the country, for the ebb and flow of movement of the American population will carry many of these cases into areas where coccidioidomycosis is unknown and unsuspected.

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## Section II

# Therapeutic And Immunologic Aspects Of Coccidioidomycosis

# The Treatment of Pulmonary Coccidioidomycosis from a Surgical Standpoint

Dermont W. Melick, M.D.

At the present time it is estimated that at least 400 patients with pulmonary coccidioidomycosis have been treated by surgery. A survey revealed that 98 had been subjected to surgical therapy by 1950.(1) A second survey in 1953 brought the total to 250 and a recent estimate has increased it to 400.

Cotton, Paulsen, and Birsner(2) have reported the largest series of surgical cases – 110. Forsee and Perkins(3) reported 50 cases from Fitzsimmons Army Hospital in Denver and surgeons in Phoenix and Tucson, Arizona, have reported 100 cases.

**Indications for Surgical Therapy**

- **I. Absolute**
    - a. Giant Cavity (over 5 cm.)
    - b. Infected Cavity
    - c. Ruptured Cavity
        - 1. Bronchopleural Fistula
        - 2. Empyema
        - 3. Nonexpansile Lung
        - 4. Persistent Pneumothorax
- **II. Probable**
    - a. Enlarging Cavity
    - b. Cavity with Hemorrhage
- **III. Possible**
    - a. Coin Lesion
    - b. Persistent Cavity

There should be little argument about the need for surgery in group I.

Cases in group II give definite warning signs of impending complications, and definitive surgical therapy is considered urgent. An enlarging cavity creates a hazardous situation. If the cavity is near the visceral surface of the lungs, rupture may occur and cause pneumothorax. Persistent bronchopleural fistula, empyema, and nonexpansile lung are also disturbing sequelae to rupture. If the patient is fortunate and the rupture is small, a pleural effusion may be the only complication. Hemorrhage may be severe in an enlarging cavity. When hemorrhage is recurrent surgical extirpation of the cavity is indicated.

The classification of a solid lesion in group III would not be acceptable to many thoracic surgeons, but most of them would consider a coin lesion an absolute indication for surgery. The reason it is not so considered here is to emphasize the fact that surgery would not be performed if the diagnosis of coccidioidomycosis could be established beyond question of a doubt. When a surgeon advises an operation for a coin lesion, he is in reality advising an operation for a lesion which he suspects to be a carcinoma. Statistics reveal that 30 percent of these coin lesions are malignant. This may be true in geographical areas outside the endemic area for coccidioidomycosis, but some thoracic surgeons in the endemic area believe that not over 5 percent of the coin lesions of patients in this area are malignant.

Removal of coin lesions is justified only after serious attempts at diagnosis have been made. Consideration of the age and the sex of the patient is important. Females under 30 years of age are less likely to have bronchogenic carcinoma than are males ove[illegible]e age of 40. Therefore, each case of pulmonary coin lesion merits individual attention before surgery is recommended.

The second possible indication for surgery is persistent cavitation. It has been the policy of most physicians and surgeons to treat the patient with a cavitary lesion conservatively. A newly discovered or small cavity should be kept under observation. Often it will disappear and surgery is unnecessary. Some cavitary lesions, however, have been observed to remain the same size for a number of years. If a cavity has persisted over a period of 12 to 18 months without closure, it is doubtful that it will close spontaneously. Cotton, Paulsen, and Birsner(2) state they have never

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*Chest surgeon in Phoenix, Arizona.*

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observed closure of a cavity that has persisted as long as 6 months. Need for surgery in such an instance will be determined by the attitudes of the particular patient and surgeon.

In deciding which course to follow, physicians should probably treat their patients as they themselves would like to be treated. Among our own colleagues in Phoenix, at least two have had cavitary lesions. They have been only too happy to settle for conservative treatment and, as far as I know, their health is quite good at the present time.

Relatively few complications following surgery for this disease have been reported. In their first 100 cases, Cotton, Paulsen, and Birsner(2) had one postoperative death and two patients in whom recavitation was noted and secondary operations were performed. In 50 cases reported by Forsee and Perkins,(3) two patients had recurrent cavitation, one developed a bronchopleural fistula with mixed coccidioidal and pyogenic empyema, and in another, postoperative spread was contralateral. The incidence of complications was higher in the 50 cases treated by the author.(1) Severe, disabling complications in the form of bronchopleural fistulas developed in two patients, with resulting empyema for which therapy was arduous, traumatic, and prolonged. Another patient had bronchopleural fistula and empyema which gradually healed following drainage of the empyema. One patient had a prolonged empyema which was gradually corrected by needle aspiration. In two patients, recurrent cavities have been apparent in approximately the same location as the original cavity. In two other patients persistent hemoptysis recurred over a period of three to nine months following pulmonary resection. Eventually the X-rays cleared, the hemoptysis stopped, and these patients have had no further difficulty. The complication rate for this group of 50 surgical cases is 16 percent. Complications may be expected to follow surgical treatment of pulmonary coccidioidomycosis as long as there is no known antibiotic or fungicidal drugs to control the etiological agent. Despite the complications, there is no doubt that surgery has a definite place in the therapy of pulmonary coccidioidomycosis.

## Conclusion

Surgical therapy is a well recognized procedure in the treatment of certain cases of pulmonary coccidioidomycosis. Surgery has been performed in approximately 400 cases at this writing.

The complications following this type of surgery have been low in the majority of reported cases. When complications do occur, they may be severe due to the lack of a fungicidal agent to assist in protecting the patient when surgery is performed.

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# Chemotherapy for Coccidioidomycosis in Man

## *——Review of Recently Employed Antifungal Agents——*

Victor D. Newcomer, M.D., Carlyn Halde, Ph.D., and
Thomas H. Sternberg, M.D.

A large variety of chemotherapeutic agents have been employed for the treatment of coccidioidomycosis. All have been uniformly disappointing. Recently a large number of antifungal agents have become available for clinical trial, and many of these have been reported as favorably influencing the course of coccidioidomycosis in man.

It is the purpose of this review to summarize some of the data in respect to the use of these compounds for the treatment of coccidioidomycosis.

### Prodigiosin

Lack (1) found that prodigiosin, a tri-pyrrole methane purple dye obtained from cultures of *Serratia marcescens*, inhibited the growth of *Coccidioides immitis in vitro* and was of protective value when given to infected animals.

Wier *et al.* (2) have used prodigiosin for the treatment of 14 patients with disseminated coccidioidomycosis. Oral, intramuscular, and intravenous preparations were employed. Five of the patients were considered hopeless at the time therapy was begun. Of the 9 remaining patients, 3 apparently recovered, 3 showed clinical improvement, and 3 showed no response. The drug has not become available for general use, and its value for the treatment of coccidioidomycosis has not been studied further.

### Aromatic diamidines

The early clinical studies of the aromatic diamidines as fungicidal agents were concerned with the use of propamidine and stilbamidine for the treatment of North American blastomycosis.(3,4) Their favorable influence on the course of this disease further stimulated the study of the aromatic diamidines not only for the treatment of blastomycosis but also for other systemic mycoses including coccidioidomycosis. Propamidine was found suitable for local use on the cutaneous lesions of blastomycosis but was too toxic for parenteral administration. Stilbamidine, however, warranted further study.

Cohen and Bos (5) found that stilbamidine (4.4' stilbenedicarboxamidine) inhibited the growth of *C. immitis in vitro* at a concentration of 75μg./ml. of medium. They treated a three-year-old Mexican boy who had disseminated coccidioidomycosis with cutaneous, pulmonary, and osteolytic lesions of the skull.(6) In addition, the patient had a coexisting pulmonary tuberculosis. He was given 1 gm. of streptomycin daily for treatment of his tuberculosis. Stilbamidine was given intravenously for 30 days starting with a dosage of 30 mg. daily which was increased to 150 mg. daily during the last 18 days. The patient succumbed, however, and at autopsy acute yellow atrophy of the liver, fatty infiltration in the brain, and a fatty infiltration of kidney tissue were found. Death was attributed to the toxic effects of stilbamidine.

Gephardt and Hanlon (7) treated a 33-year-old white male who had disseminated coccidioidomycosis with lesions involving the skin and laboratory evidence of meningeal involvement. A course of stilbamidine (1.95 gm. in 27 days) was given approximately two years after the onset of his illness. All the cutaneous lesions healed except one. Although laboratory studies showed improvement in the condition of the spinal fluid 12 months after stilbamidine treatment, residual abnormalities suggest a persistence of meningeal involvement.

The total amount of stilbamidine which can be given has been limited by the invariable development of a complication most frequently involving the trigeminal nerve. This has consisted of progressive sensory changes manifested by paresthesia, anesthesia, hypalgesia, and numbness. On the other hand, 2-hydroxystilbamidine apparently

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does not cause neuropathy, and the use of this drug for treatment of North American blastomycosis has completely replaced stilbamidine.

Snapper *et al.* (9) have reported their experience with the use of 2-hydroxystilbamidine in the treatment of seven patients with proved disseminated coccidioidomycosis. Individual total dosages varied from 8 to 23 gm. A review of the clinical course, physical findings, and laboratory data in all these cases revealed no evidence of a curative effect of the drug on disseminated coccidioidomycosis since evidence of low grade infection persisted. In at least five of the seven patients, 2-hydroxystilbamidine therapy was followed by more or less marked suppression of the disease. This was manifested by progressive subsidence of complaints, gain in weight, and a resumption of normal activities. It was their impression that after the completion of treatment the acute infection of *C. immitis* had been so markedly reduced in activity that a condition designated as commensalism had resulted.

Nitritoid reactions were noted when high concentrations were administered intravenously. These were avoided by decreasing the concentration per unit time of injection. Formication was also noted to be dependent on rapidity of injection. After receiving 10 to 14 gm. of 2-hydroxystilbamidine, patients complained of malaise, weakness, headache, muscle ache, nausea, and vomiting. Gordon *et al.* (10) on the basis of Snapper's findings undertook a study of the inhibitory potential of this drug against *C. immitis in vitro* and in mice. Complete inhibition of growth *in vitro* was obtained when the organism was added to media containing 100µg. per milliliter of 2-hydroxystilbamidine. The administration of 2-hydroxystilbamidine in just below toxic levels to mice infected with *C. immitis* failed to demonstrate any beneficial effect. The drug did not reduce or delay mortality in the treated group. Their studies suggested that the dosages required for therapeutic effect would be too large to be practical.

The chemical similarity of the aromatic diamidines to diethylstilbestrol suggested to Curtis and Harrell (11) the possibility that diethylstilbestrol might also have an effect on blastomycosis. They studied the fungistatic effect *in vitro* of diethylstilbestrol, stilbamidine, and other sex hormones on the growth of *Blastomyces dermatitidis*. Diethylstilbestrol and methyltestosterone both showed promise of inhibiting the organism and were considered worthy of further investigation. The authors subsequently treated two male patients with cutaneous blastomycosis with 3 mg. of diethylstilbestrol daily. There was a complete disappearance of their lesions in three to four months. The *in vitro* fungicidal spectra of four stilbene derivatives, stilbamidine, propamidine, pentamidine, and diethylstilbestrol, were presented in a subsequent and more detailed paper.(12) Methyltestosterone and isonicotinic acid were also tested against *C. immitis* but showed no fungicidal activity in concentrations as high as 1 mg./ml. of media. Diethylstilbestrol was found to inhibit the growth of *C. immitis* effectively at a level of 10µg. per milliliter of medium. On the basis of the *in vitro* activity of diethylstilbestrol against *C. immitis* and the successful use of this compound for the treatment of North American blastomycosis, Piper *et al.* (13) used diethylstilbestrol for the treatment of two male patients with disseminated coccidioidomycosis. They were given daily intramuscular injections of 100µg. of diethylstilbestrol for 63 and 100 days respectively. No effects on the cutaneous lesions were observed. Both patients developed gynecomastia and decreased libido, but to no greater extent than was observed with the more conventional dose. However, 12 weeks after cessation of therapy the sperm production was still either greatly decreased or totally absent.

Lamb, (14) using a pour plate *in vitro* method, found testosterone, methyl-testosterone, and testosterone propionate to be among the more fungistatic of over 50 steroid compounds which they tested. Concentrations of 0.01 percent produced complete fungistasis of *Histoplasma capsulatum* and *B. dermatitidis* and a partial to complete fungistasis of *C. immitis*. Testosterone propionate did not, however, alter the course of experimentally produced coccidioidomycosis in mice, and the sera of mice receiving testosterone propionate in oil could not be demonstrated to possess fungistatic activity.

Lamb has treated one patient, a 29-year-old Negro, who had generalized coccidioidomycosis. This patient's complement fixation test was 2 plus at a 1:256 dilution, and the skin test with coccidioidin was negative. For a period of approximately one year the patient was given methyltestosterone varying in dosages from 100 to 250 mg. per day. In addition, the patient was given two

7.7 grain tablets of meth-dia-mer sulfonamide three times a day. At the end of this time the patient gained 40 pounds, was in good general health, and his disease was considered arrested. During this period, however, there was only slight improvement in the complement fixation test but his skin test showed 1 plus reaction to coccidioidin in a dilution of 1:100. Lamb felt that a certain level of testosterone must be maintained to obtain a curative effect. He suggested at least a minimal dose of 100 mg. per day.

Hoffman and Doerner (15) reported on a 46-year-old Filipino-American shipyard worker who had disseminated coccidioidomycosis with mono-articular involvement, for which prolonged combined estrogen and androgen therapy was used. Chloroquine was given for one month prior to the above mentioned therapy in a dosage ranging from 2 gm. to 0.625 gm. per day. The drug was decreased and eventually discontinued because of the patient's persistent nausea. However, in retrospect the authors felt that the recovery of the patient seemed to start just before the chloroquine was discontinued. The patient then received testosterone cyclopentopropionate, 200 mg. intramuscularly twice a week; ethinyl estradiol by mouth, 0.02 mg. three times a day. In addition, the patient was given 2.5 mg. of trypsin twice a week.

These medicaments were administered for a period of three months. During this period there was a steady increase in body weight, decrease in leukocytosis, decrease in sedimentation rate, increase in hemoglobin and hematocrit, a complete closure of the wounds about the knee, and a dramatic restoration of general well being.

### Isoniazid

The striking effectiveness of isoniazid and related compounds on *Mycobacterium tuberculosis* coupled with the many points of similarity between tuberculosis and coccidioidomycosis prompted Tager (16) to investigate the action of isoniazid and iproniazid *in vitro* and *in vivo* against *C. immitis*. They found no evidence that iproniazid inhibited *C. immitis* at 1,000, 100, and 10 μg. per milliliter of media. Isoniazid, however, inhibited the growth of *C. immitis* at 1,000 μg. but was ineffective at 100 and 10 μg. per milliliter of media. No evidence of protection against *C. immitis* was obtained from *in vivo* studies conducted in mice.

Fostvedt *et al.* (17) treated three patients who had coccidioidomycosis with isonicotinic acid hydrazide on the basis that coccidioidal granulomata are histologically typical granulomata with tubercle-like formations, indistinguishable from those seen in tuberculosis. All patients were Negro males, one with a progressive pneumonitis of the left lung and one with disseminated coccidioidomycosis confined evidently to two subcutaneous granulomata which developed into abscesses. The third patient had a localized pneumonitis in the lower right lobe. The first two patients were considered to have a serious prognosis. They were given 100 μg. of isonicotinic acid hydrazide three times a day. In addition, a multivitamin preparation was given for the purpose of avoiding deficiency sequelae produced by the metabolic competition between isonicotinic acid hydrazide and menadione, pyridoxine, niacin, and possibly other B-complex factors. The medicaments were administered over a period of four months at the end of which time both patients had gained weight and all evidence of disease had disappeared.

The last patient was not ill and a trial of minimal therapy was decided upon. Fifty μg. of isonicotinic acid hydrazide was given three times a day over a period of three weeks. The density observed in the X-ray films of the chest decreased, and the patient became asymptomatic. One month later, however, the patient was rehospitalized for further study and the sputum was found to contain *C. immitis*. The patient was given 100 mg. of isonicotinic acid hydrazide three times a day for a period of about three months. At the end of that time the lungs were clear, and the sputum specimens were negative. In all three of the cases, the most striking feature was the return of appetite or increase in appetite associated with isonicotinic acid hydrazide therapy. There was an average weight gain of four and one-half pounds per patient per month.

Since the writing of their paper Fostvedt *et al.* have treated three additional patients in whom an early diagnosis of pulmonary coccidioidomycosis in variable degree has been made. These patients were responding and recovering satisfactorily at the time of the report.

### Ethyl vanillate

Ethyl vanillate (ethyl 4-hydroxy-3-methoxy-benzoate) was first developed as a by-product in the manufacture of sulphite pulp during the course of a research program on waste disposal in the paper industry.

*In vitro* studies by Christie *et al.* (18) indicated that the growth of *H. capsulatum* was inhibited by ethyl vanillate in a concentration of 20 mg./100 ml. in plasma fortified media incubated at 37° C. McVickar (19) found the growth of *C. immitis* to be inhibited in concentrations of 10 to 30 mg. per milliliter of media.

Christie's promising experience in the treatment of histoplasmosis prompted Fiese *et al.* (20) to evaluate the drug for the treatment of disseminated coccidioidomycosis. They treated eight patients with ethyl vanillate but in retrospect concluded that a really adequate concentration of ethyl vanillate was maintained in only two of the patients. It seemed to be more than coincidental that these two patients responded best, although neither of them appeared to be completely cured of the disease. A third patient seemed to have been helped by ethyl vanillate, but evaluation of his case was difficult because the blood concentration of the drug was somewhat less than optimal. For all practical purposes, the other five patients were not treated with the drug. Four more patients with disseminated disease were reported in the addendum. Three were definitely helped by the administration of ethyl vanillate.

The concentration of ethyl vanillate lethal to *C. immitis* is estimated to be between 20 and 40 mg. per milliliter of blood. Although such concentrations are relatively non-toxic, they are obtained with considerable difficulty since the drug can be given only by mouth. The patient must take from 32 to 48 gm. daily. This is possible only for a patient who is alert, cooperative, and free of nausea and vomiting. It is practically impossible to attain a constant, therapeutically effective concentration on the patient who needs it most, namely those with coccidioidal meningitis or those with fulminating, generalized coccidioidomycosis. The administration of the drug by means of a stomach tube has been found unsatisfactory.

Fiese felt that the patients who were most likely to be helped by ethyl vanillate were those with relatively early progressive lesions of the skin, subcutaneous tissue, bones, or viscera. Although they may be weak and losing weight and have a high erythrocyte sedimentation rate and an increasing complement fixation titer, they are still in fairly good general condition, mentally able to cooperate, and physically able to eat and retain food. Less likely to be helped are those patients with coccidioidal meningitis or with wide-spread dissemination who are too ill to cooperate. Fiese *et al.* felt that ethyl vanillate is a worthwhile therapeutic agent which may be curative in selected cases of the disease.

**Nystatin**

Nystatin, an antifungal antibiotic, was first isolated by Hazen and Brown in 1949 from cultures of *Streptomyces noursei*.(21,22) *In vitro* studies indicated that it effectively inhibited a large variety of nonpathogenic and pathogenic fungi including *C. immitis* in concentrations of 1.56 to 6.25 μg./ml. of media.(23) The course of coccidioidomycosis in mice was found to be significantly altered by nystatin as manifested by longer periods of survival and in some instances by apparent biological cure.(24-28)

Amounts of the drug calculated to be within the therapeutic range could be administered to various animals for long periods of time with minimal evidence of toxicity. Evaluation of nystatin, therefore, as a therapeutic agent for the treatment of coccidioidomycosis in humans seemed warranted.

Newcomer *et al.* (29,30) presented their observations after treating five patients with disseminated coccidioidomycosis. Two patients, a 13-year-old Mexican girl and a 27-year-old Negro male, who had meningitis due to *C. immitis* were treated with nystatin, but there was no evidence of clinical improvement. It was concluded that nystatin does not gain access to the spinal fluid from the blood since no appreciable levels of nystatin were detected in the spinal fluid.

One Negro male with subcutaneous abscesses and a lytic lesion in a rib was given 400,000 units of nystatin intravenously daily for a period of 4 months. At the end of this period the lesions had healed, and at the present time, one and one-half years later, the patient shows no evidence of active disease.

A second Negro male with multiple subcutaneous pulmonary and osteolytic lesions was given 800,000 units of nystatin daily for a period of 4 months. At the end of this time the patient left the hospital against medical advice, and he was not seen again until he re-entered the hospital approximately one year later. He stated that approximately two months after leaving the hospital all drainage from the cutaneous sinuses had ceased. At the time of re-admission the patient presented two draining sinuses from which *C. immitis* could be cultured.

The patient again responded to 200,000 units of nystatin, given intravenously daily for a period of two months, with healing of the sinus tracts. Long range followup on this patient has not been possible.

The third patient was a 23-year-old white male with diabetes mellitus. He had a right bronchopleural fistula and a sinus tract draining into the pleural cavity from which *C. immitis* could be isolated. He was given 200,000 units of nystatin intravenously for a period of three months, following which he underwent a three-stage extrapleural pneumonectomy. At the present time, a year and a half after beginning nystatin therapy, the patient is in good health, but *C. immitis* can be cultured from a draining sinus tract which persists in the surgical wound of the pneumonectomy.

Nystatin is not readily absorbed from the gastrointestinal tract and the intravenous administration of the drug is associated with nitroid-like reactions consisting of chills, fever up to 104° F., convulsions, and shock-like states. Laboratory studies, however, have failed to demonstrate any evidence of toxicity but because these alarming reactions sometimes occur, the intravenous use of nystatin does not seem warranted.

Tentatively these results appeared to be comparable to, but no better than, those which were reported with ethyl vanillate, 2-hydroxystilbamidine, and prodigiosin.

## Amphotericin

Two new and closely related antifungal antibiotics, amphotericin A and amphotericin B, have recently been isolated from an unidentified species of *Streptomyces* first obtained from the soil of the Orinoco River region in Venezuela.(31,32) *In vitro* these antibiotics inhibit a wide variety of non-pathogenic and pathogenic fungi in concentrations of 0.5 to 12.5 µg./ml. They are not, however, effective against bacteria at levels as high as 50 µg./ml. Amphotericin A is effective against a wider variety of fungi but amphotericin B is several times more active against pathogenic fungi. The toxicity of these antibiotics is relatively low when they are administered intraperitoneally in mice. Both of these antibiotics favorably influence the course of experimental infections produced by *C. immitis* in mice.(33) Amphotericin B was more effective regardless of the route of administration. It also showed a protective and, in some instances, a biological curative effect when administered daily, either orally or intraperitoneally, to mice experimentally infected with *C. immitis*.

In a detailed study Halde *et al.*(34) found that the oral administration of amphotericin B dramatically influenced the course of coccidioidomycosis in mice. This was manifested by longer periods of survival and in some instances biological cures. In an attempt to further evaluate the therapeutic value of amphotericin B, experiments were done in which the drug was withheld from groups of mice until they appeared moribund. Within three days after drug administration was begun, the condition of the mice improved so that their appearance and activity resembled that of the normal controls. Cultures taken at autopsy still revealed the presence of *C. immitis* in the majority of the animals. However, no organism could be cultured from 20 percent of the animals.

The amount of amphotericin B in the serum of mice, following various routes of administration, was determined and in no instance was the serum level high. The highest serum levels were obtained following massive intraperitoneal administration. It is of great interest that the majority of mice with experimental coccidioidomycosis responded dramatically to the oral administration of amphotericin B in a daily dosage of 600 mgm./kg. when the serum level was low.

One patient with disseminated coccidioidomycosis was treated with oral preparations of amphotericin B. He was on various dosage schedules up to 10 gm. a day. Numerous blood studies directed towards determining the amount of amphotericin B in blood showed that only detectible amounts were present. No response to amphotericin B was observed during this period of therapy, and it was concluded from this patient and from one other with cryptococcosis that the drug was not absorbed from the gastrointestinal tract of man in sufficient quantity to significantly influence the course of coccidioidomycosis.

## Discussion

The growing list of medicaments which have favorably influenced the course of disseminated coccidioidomycosis in both animal and man appears to be most encouraging and has proved a tremendous stimulus for the continuing and increasing efforts directed toward finding and testing newer compounds. The number of compounds possessing fungicidal properties *in vitro* throughout a wide range of concentrations seems limitless. For example, Alexopoulos(35) has shown that as many as 56 percent of all cultures of actinomycetes isolated from the soil possessed some antifungal properties. Relatively few of the compounds, however, favorably influence the course of coccidioidomycosis *in vivo* in experimental animals and even fewer possess characteristics which permit administration to humans.

The ease of evaluating the effect of such medicaments on the course of coccidioidomycosis in man is made difficult by many factors. These include a need for a thorough understanding of the natural course of the disease, adequate controls and material, and sufficient followup periods, as well as unbiased observations. When dealing with humans the majority of these requirements cannot be fully met, and thus conflicting views and opinions are to be expected. It is agreed, however, that a suitable medicament for the treatment of coccidioidomycosis is not available today. There is question as to whether even a mildly effective drug is available. On the other hand, there is no doubt that certain patients with sufficiently acute cases of coccidioidomycosis to justify their inclusion in clinical investigative programs have frequently responded favorably and in some instances dramatically to a wide variety of medicaments.

This same type of phenomenon has been observed in the treatment of other systemic fungus infections. For example, Harrell *et al.*(36) first enthusiastically supported the curative value of stilbamidine and/or 2-hydroxystilbamidine for the treatment of North American blastomycosis. After a three-year followup period of their cases, however, they speculated that perhaps the patients' natural immunologic response to the disease process governed the favorable or unfavorable results of the present-day stilbamidine treatment more than anything else.

In an effort to explain similar findings currently encountered in the treatment of coccidioidomycosis it would seem worthwhile to re-examine critically the serious prognosis (30-50 percent fatality) generally associated with disseminated disease. On the other hand, optimal supportive measures which are usually given along with new investigative drugs and are directed toward the development of maximal host resistance may play a more vital role than had formerly been expected. This treatment coupled with even mildly effective fungicides may be all that is needed to produce a favorable outcome in this disease.

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# Therapy of Coccidioidomycosis

Marshall J. Fiese, M. D.

The title of this panel discussion is almost misleading, for one might think that we have something exciting and definitive to say about the therapy of coccidioidomycosis. In general, our knowledge of *Coccidioides immitis* and the varied disease it causes is precise and certain. Most of the subjects discussed at this conference have the authority of accurate and complete scientific data. The treatment of coccidioidomycosis, on the other hand, is a subject to be approached with humility. Our knowledge concerning medical therapy has lagged far behind our knowledge of other aspects of the fungus *C. immitis*. We do not have the answer at this time, although research concerning treatment continues apace in several interesting directions.

The paradoxical situation of intimate acquaintance with a disease and the embarrassing confusion concerning what to do about it are, of course, not unique. Etiologic knowledge and pathologic understanding are usually precursors of therapeutic ability. For instance, much was known about diabetes before insulin was even discovered. Therapy is often the last frontier.

It is a sobering experience to study and report with cautious optimism a new therapeutic agent for coccidioidal disease and then to read an ancient article, with almost the same words and results, about a drug now long discredited. We have only to see the old accounts of occasional "cures" with antimony and potassium tartrate, sodium iodide, colloidal copper, or a dozen others – "cures" as dramatic as any we can relate now – to realize the vagaries of coccidioidal infection. Helen Mackler has appropriately called coccidioidomycosis "a disease without a natural history."(1) The course of the untreated disease is so erratic and unpredictable that evaluation of any drug is exceedingly difficult. Possibly a large series of experimental drugs, perhaps larger than any one of us will see, will be necessary to give the final answer. In the meantime, we must continue to try the most promising agents; we must look at our results honestly and a little incredulously; and we must not take ourselves too seriously.

In my opinion, an honest appraisal of 60 years of various forms of treatment compels these observations:

(1) The course of acute, fulminating, disseminated coccidioidomycosis has never been altered by any form of therapy, past or present.

(2) Meningitis, too, has probably not been affected by treatment, at least, not often enough for us to draw any conclusions.

(3) Dramatic improvement of chronic, disseminated lesions has coincided with treatment by several methods and with many drugs. It has also occurred with no treatment whatsoever.

(4) Drugs which have been apparently curative in some cases have failed in other similar cases.

(5) Apparent eradication of disseminated lesions may be followed 10 or 15 years later by severe and even fatal recurrence.

These disquieting observations must temper any discussion of treatment of disseminated coccidioidomycosis. Incredulous realism, though perhaps not complete therapeutic nihilism, is in order.

The best control of coccidioidal disease would be its prevention. Prophylaxis could conceivably be accomplished in two ways: (1) by preventing contact between the fungus and susceptible persons, and (2) by inducing artificial immunity in the prospective host. The first possibility would involve eliminating either the fungus or susceptibles from endemic areas. It is obvious that susceptibles cannot be kept out of the endemic areas, since these sections are experiencing some of the most rapid population growth in the United States. The most highly susceptible races are not likely to avoid the endemic areas in view of the demand for agricultural laborers. It is even more absurd, with our present knowledge, to hope to eliminate the fungus from the vast reaches of its desert habitat. This aim can be approximated on a small, local scale by dust control measures, as C. E. Smith and his colleagues demonstrated so clearly in the air fields

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of the San Joaquin Valley during World War II.(2) This approach is highly proper for protecting the short-term resident in an endemic area, but it seems that such measures would only postpone the infection of the permanent inhabitant.

The most promising way of preventing infection is the production of artificial immunity in susceptibles. Of all the problems concerning coccidioidomycosis, I believe this is the field most worthy of research. We might predict that definitive control of coccidioidal infection will come with an effective means of vaccination. Preliminary observations have already been made by Vogel, Fetter, Conant, and Lowe,(3) and by Friedman and Smith.(4) If the peoples of the southwestern United States – particularly those of susceptible races – and those newly arrived for agricultural work could be effectively vaccinated against *C. immitis*, the problem of coccidioidomycosis would be essentially solved.

Any discussion of the treatment of coccidioidomycosis revolves around the treatment of its disseminated form. Usually the primary infection requires no therapy other than the symptomatic measures one would reasonably use in any acute, benign respiratory disease. If an effective and safe chemical agent were available, it might also be used for primary infections in members of the susceptible races such as Negroes and Filipinos in an attempt to prevent dissemination.

A long list could be made of the drugs which have been tried in the treatment of disseminated coccidioidomycosis since the first patient was treated 65 years ago. At the present time, research concerning therapy follows two main patterns. In some institutions, particularly in the East and Midwest, a number of fungicides are being tried against various mycoses, and coccidioidomycosis often appears as one among several such diseases under experimental treatment. Here in the Southwest, research tends to be aimed more specifically at coccidioidal disease, and is sometimes more empiric.

One problem concerning the experimental use of fungicides against *C. immitis* is that we are not always sure that the *in vitro* effect parallels the *in vivo* effect. The parasitic and saprophytic phases of *C. immitis* are quite distinct; perhaps they act like different organisms as far as their pharmacological properties are concerned. It may be that with a certain drug there is no relationship between the concentration required to kill the mycelial form in the test tube and the spherule form in the tissues. Methods to perpetuate spherule propagation *in vitro* may therefore be required in order to test the effectiveness of drugs.

Several fungicides currently available probably have a mild *in vivo* effect against *C. immitis*. It is well known that many patients, particularly Caucasians, handle disseminations well. They seem to strike a balance with the infection. Organisms are still harbored, the sinuses drain periodically, the complement fixation titer remains elevated, but the Caucasian patient gains weight, feels well, and lives a relatively normal life. It is my feeling that in certain cases of slowly progressive dissemination in dark-skinned patients whose natural resistance is notoriously poor, enough help should be given by one of the modern therapeutic agents to allow them to strike such a balance with the disease that it may be walled off and a sort of "peaceful coexistence" be achieved.

It is not my purpose to extol any particular drug, but rather to cite several which illustrate these general concepts. My associates and I have reported elsewhere our early observations concerning the use of ethyl vanillate. (5,6) Cohen has also described its *in vitro* effect (7) and its clinical use. (8) Dennis and Hansen reported its use in children, with results similar to ours. (9) I will mention two illustrative cases.

Our first patient to be treated with ethyl vanillate was a Filipino farm laborer whose disease we had followed from the time of severe primary pneumonia to dissemination three months later. During this time, rapidly progressive abscesses of the back and extremities, and roentgenographic evidence of bone destruction in the extremities, spine, and skull were observed. Our first timid doses of ethlyl vanillate were so small that the blood level was negligible and new lesions continued to appear. With bolder doses the lesions healed, the patient gained 20 pounds in weight, his sedimentation rate fell to normal, and his complement fixation titer regressed (fig. 1). Now, six years later, he is in good general health and is able to work, although small lesions still recur occasionally in a finger and a toe.

Another Filipino man came to us with an ugly coccidioidal granuloma of the whole left hand and wrist of several years duration. The small bones were distorted and fused into a shapeless con-

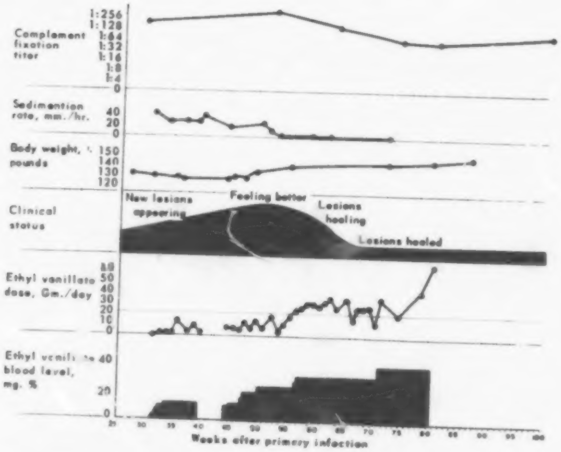

**Figure 1. Clinical course of a patient with chronic disseminated coccidioidomycosis treated with ethyl vanillate.** ***Courtesy of Stanford Medical Bulletin.***

glomerate. His fingers were useless. The soft tissues were a foul, suppurative mass, swimming with spherules of *C. immitis*. We advised amputation, but when he refused, we tried ethyl vanillate. Within six days the skin began to heal and thereafter all the sinuses slowly closed. When seen recently, his hand was covered with soft, supple, intact skin. Useful motion had returned to the fingers. Complement fixation titer had fallen from 1:256 to 1:64. We were glad he had talked us out of amputation, for he now has a useful hand.

Two serious criticisms must be made of ethyl vanillate. First, it is a difficult drug to take. Even a small adult must take about 50 gm. per day or 25 half-gram capsules every six hours. Secondly, it has shown no beneficial effect in the patients who need it most — those with coccidioidal meningitis or acute fulminating dissemination. Usually those who are very ill cannot tolerate the prodigious doses required. It is irritating to the stomach, causing nausea and vomiting in patients who are already gravely ill.

Ethyl vanillate appears to be most useful in cases of slowly progressive disseminated coccidioidal disease. Nevertheless, its range of usefulness is narrow at best, for most patients are either not sick enough to require it or too sick to tolerate it. When something better is available, it will not be difficult to discard ethyl vanillate.

My experience with the stilbene derivatives has been limited and disappointing. We treated several patients with aminostilbamidine, but the only certain result was that at autopsy the tissues were stained bright yellow.

We have not used oral or intramuscular nystatin. Intravenous nystatin was not given a fair trial because we stopped its administration after several attempts with tiny initial doses caused alarming febrile reactions.

Recently I have used amphotericin B, an antibiotic derived from a species of *Streptomyces* isolated from soil on the bank of the Orinoco River in Venezuela, in three cases of disseminated coccidioidomycosis. There was apparent remission of the disease in each case. One case report (10) concerns a young white man who had a chronic disfiguring granuloma of the face for seven years. In January 1956, he suffered a severe exacerbation. The whole right side of the face was inflamed and edematous, and sinuses discharged on the skin, into the nares, and into the mouth. The lips became thick and boggy and the voice, hoarse. The cosmetic defect was unsightly in the extreme (fig. 2).

Four days after starting amphotericin B in doses of 2.4 gm. daily (four capsules every eight hours), he telephoned me excitedly to report that the lesions were drying up. I was skeptical, but when I saw him it was evident that he spoke the truth. Treatment was continued for three months. Disfiguration from scar tissue was still extensive, but acute inflammation and edema subsided, and sinuses closed. Ten months later another exacerbation responded to amphotericin B, but more slowly. I suspect that the organism may be developing resistance to the drug, if indeed the apparent response was anything more than coincidence.

In view of what was said at the beginning of this discussion, I might well consider these observations as anecdotes rather than as proof of anything. But we can be realistic without being pessimistic. Adequate therapy of other diseases has come from beginnings as uncertain as these. The meager results we already have may at least demonstrate that coccidioidomycosis is not necessarily incurable. Unfortunately, as far as research is concerned, coccidioidal disease is mainly of regional interest, and therefore has not been given a high priority in many of our great academic and pharmaceutical research institutions. If it were as ubiquitous as lobar pneumonia or poliomyelitis, I am sure that the

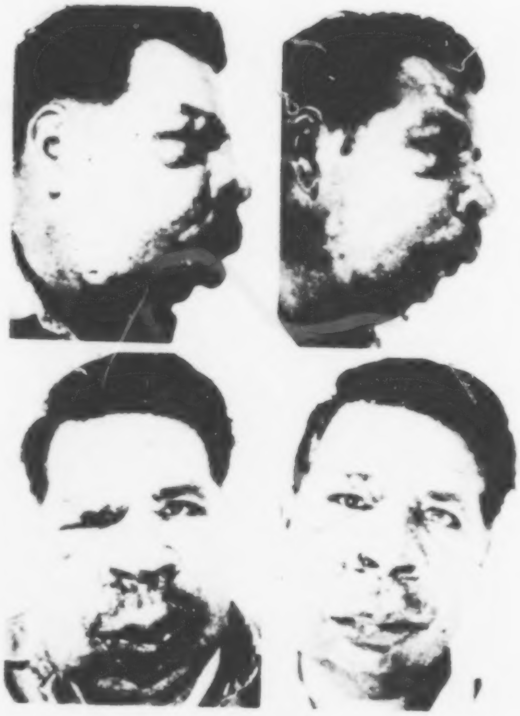

Figure 2. Coccidioidal granuloma of the face of seven years duration. LEFT - before treatment there were great edema, inflammation, and draining sinuses. RIGHT - after treatment with amphotericin B there was still extensive scar tissue, but edema and acute inflammation subsided, and sinuses healed. *Courtesy of California Medicine.*

answer would have been found before now. Perhaps the westward migration of the American people and the vast influx of new individuals into the endemic areas will stimulate others to intensify their research concerning coccidioidal disease.

As I see it, from the standpoint of the practicing physician in an endemic area, the problems concerning the treatment of coccidioidal infection which must be solved are these, in ascending order of importance:

(1) Treatment of severe primary pulmonary infections.

(2) Treatment of disseminated coccidioidomycosis, particularly of meningitis and acute fulminating dissemination.

(3) Effective vaccination against *C. immitis.*

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# Therapy of *Coccidioides immitis* Infections

Robert Cohen, M. D.

My interest in "Valley Fever" was first aroused eight years ago when I was full time chief in pediatrics at the Kern General Hospital in Bakersfield, California, which is situated in an area endemic for *Coccidioides immitis*. There I encountered a two-year-old boy with coccidioidal meningitis and discovered that there was no hopeful therapy to offer except ampules of iodine with salicylates.

Later cycloheximide,* an antibiotic derived from *Streptomyces griseus*, which inhibits *C. immitis* at a concentration greater than 1,000 mg./ml., was called to my attention. It was a moderately toxic drug – 20 mg. would induce emesis in over 50 percent of the patients. Cycloheximide was given a two-year trial and the results were presented at the American Medical Association convention in 1950 at San Francisco.(1) A few of the patients with disseminated cases who received this drug are still alive but some could not tolerate large doses and many failures resulted. However, cycloheximide did prove the important point that a severely disseminated case of coccidioidomycosis could be entirely cured.(2) An autopsy, performed on a patient who had received cycloheximide therapy and had later died of a neuroblastoma, showed that all coccidioidal lesions were completely healed.

In 1951, I reported(3) that sodium caprylate, a fatty acid, inhibited *C. immitis* at 19 to 150 mg./ml.; fradicin, an antibiotic derived from *Streptomyces fradiae*, inhibited *C. immitis* at 12.5 mg./ml.; thiolutin, an antibiotic with a high sulfur content derived from *Streptomyces alba*, inhibited *C. immitis* at 13 mg./ml.; and ethyl vanillate, a chemical derived from wood pulp, inhibited *C. immitis* at 30 mg. percent

Although sodium caprylate proved unsatisfactory when administered intravenously, intramuscularly, and orally, it still remains acceptable in 20 percent solution for wet dressings and for irrigating open sinuses. Fradicin was too toxic. Thiolutin was only slightly soluble in alcohol and because only a small amount could be given, it had to be discontinued. I have used ethyl vanillate since 1951 and it has not let me down. Despite the fact that it has a pungent taste and has to be given in large dosages over an extended period of time, I have received favorable results in many cases of disseminated coccidioidomycosis, especially in Negro, Filipino, and Chinese patients.

For two years the Wisconsin Institute for Paper Chemistry sent me bulk quantities of ethyl vanillate, which I melted on my wife's kitchen stove and poured into thousands of gelatin capsules. This crude procedure often left a bitter-tasting material on the outside of the capsules which made them unpleasant to take in large amounts. Finally, a pharmaceutical company put the drug in acceptable capsules and made it much easier to give.

Experience has taught me to start therapy with ethyl vanillate rather slowly and gradually work up to as many as 60 to 70 0.5 gm. capsules per 24 hours. One has to "baby his patient" with this drug, giving food before each administration and milk during the course of it. Some doctors who have written me of its so-called toxicity during the taking did not consider this need. Of more than 40 patients with disseminated cases who received ethyl vanillate over a five-year period, 70 percent have been helped and have returned to their occupations or schools. One patient, a Negro woman who had coccidioidal meningitis with a positive spinal fluid culture, took 30 gm. of ethyl vanillate daily for 18 months. More than five years have passed now, and she has gained over 100 pounds and still picks cotton in Delano, California. Ethyl vanillate has merit and will help in disseminated cases until a speedier-acting and better drug is found. The research department of a large pharmaceutical company made some studies on vanillic

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*The specific product used was Acti-dione which is manufactured by Upjohn Co., Kalamazoo, Mich. The use of trade names does not constitute endorsement by the U. S. Public Health Service.

esters such as isopropyl, butyl, isobutyl, and secondary butyl vanillate. They suggested that antimicrobial action is increased by lengthening the aliphatic chain and ethylation of the ring.

Some patients will get well in any event. One of mine, a Negro child, had a complement fixation titer of 4 plus in the dilution of 1:1024 before therapy consisting only of vitamins and rest. Today her complement fixation titer is 2 plus in 1:4 dilution.

I reported(5) that chloromycetin failed to inhibit *C. immitis* at 2,000 mg./ml. and in 1952 I reported that stilbamidine(6) inhibited *C. immitis* at 75 mg./ml. The latter drug was used in 6 cases and unfortunately one of the patients developed acute, yellow atrophy of the liver and died. Although there have been favorable reports on the use of this drug against North American blastomycosis, I have no regard for its usefulness in combating *C. immitis*.

Rimocidin, a drug obtained from the culture broths of *Streptomyces rimosus*, was found to inhibit *C. immitis* at 25 mg./ml.(6) However, because this drug was hemolytic for human red cells at 30 mg./ml., it was discarded. *In vitro* studies with atabrine and isonazides were discontinued when a heavy overgrowth with *C. immitis* occurred.

Para-aminobenzoic acid was reported in 1953(8) as a fungicide and found to inhibit *C. immitis* completely at 1,500 mg./ml. When it was tried on two severely disseminated cases, the patients' erythrocyte counts dropped quite low and no hopeful responses were seen.

In 1952, a study on nystatin(9) showed that it could inhibit *C. immitis* at 25 mg./ml. Whereas Hazen(9) used N/50 HCL to get the drug into solution, I found it quite soluble in propylene glycol, which also inhibited *C. immitis* at 70.500 mg./ml.(9) The drug was not absorbed systemically and was therefore dropped. However, it is commercially available and has proved effective for the treatment of other infections.

Diethylstilbestrol, a stilbamide derivative, inhibited *C. immitis* at 1 mg./ml.(10) It was hard to evaluate the drug's true value in two disseminated cases although it seemed to prolong the life of these patients either because of its fungicidal property or by its nitrogen-retaining ability.

A fungicidal drug labeled NF-254 inhibited *C. immitis* at 11 mg./ml. when given to fowl.(11) They became blind and the central nervous systems were affected so that many became opisthotonic. The drug appeared to be far too toxic to warrant further experimentation.

Sometimes streptomycin sulfate is given intrathecally to patients during the interval before a laboratory report on the differential diagnosis between coccidioidal and tubercular meningitis has been received. Often, when the patients appear to brighten up for a while, the infection turns out to be of coccidioidal origin. Streptomycin completely inhibited *C. immitis* at 15,000 mg./ml.(12) but this is not sufficiently concentrated to do much good since the amount that can be given is limited. Cases of coccidioidal meningitis may be prolonged for two years and this drug may offer some holding power.

Some plants and plant products – such as extract of buttercup – inhibit fungi. I have used oil of sassafras and found that it inhibited *C. immitis* at 500 mg./ml.(13) in both primary and disseminated cases. No harmful effects were noted and it might help in some small way.

The sodium salt of cinnamic acid(13) has been reported to inhibit *C. immitis* at 30 mg./ml. when injected intramuscularly or intravenously in 5 percent solution. In my study I was unable to get higher concentration than 4 mg./100 cc. in the blood in the two cases. I hope to try higher concentrations soon. I am also trying oral dosages of 250 mg. every 4 hours in a few cases. This drug, which appears to have no side effects, will be studied further.

Nicetin CMA-37, an antibiotic related to chloromycetin, was found to inhibit *C. immitis* at 0.0025 molar concentration.(14) It was strongly stressed that red blood cells would spill into the urinary tract during therapy but that this would stop soon after the drug was discontinued. I tried this drug on four patients with disseminated cases and they all apparently responded well. All had hematuria while taking the drug but it disappeared as predicted. One small girl, who had a coccidioidal granuloma below the larynx and wore a tracheotomy tube for six months, was given 100 mg. instead of the stated dosage of 250 mg. in three courses of 5-day trials. Her complement fixation dropped and her gross hematuria slowly let up when the drug was stopped. One year later this child had a severe attack of croup. She was exhausting herself even in a croupette with oxygen and another tracheotomy was done. A bronchoscopic examination at that time showed complete resolution of the coccidioidal granuloma seen a year earlier.

In 1956 Dr. Keith Maddy informed me of a new plant fungicide, N-trichloromethyl tetrahydrophthalimide, that he was using in coccidioidal studies. In testing this drug's potentialities, I found that it inhibited *C. immitis* at 2.5 mg./ml. I sent the drug to the research laboratories of a large pharmaceutical house and requested that they make up various dilutions with coded numbers. After the dilutions were returned, I plated Petri dishes and found that the first batch of 48 plates inhibited *C. immitis* at 100 mg./ml. Because penicillin and streptomycin were added to the plates I requested a repeat study without these two antibiotics. I am not aware of Dr. Maddy's *in vitro* study on this drug but I think it will show a very low inhibiting concentration. A preliminary toxicology study of N-trichloromethyl tetrahydrophthalimide was reported in 1946.(15)

It has become obvious that many drugs have fungicidal properties toward *C. immitis* but few can be used therapeutically. Such side reactions as undue emesis, hematuria, loss of blood, and severe, systemic damages to other organs are factors to be considered in the evaluation of drugs. The inability to get sufficiently high blood concentrations to exert an effect or to cross the meningeal barrier is a challenge worthy of acceptance.

We know that men have their weaknesses, bacteria have theirs, and surely *C. immitis* has its weakness, too. It is just a matter of time until the drug or chemical is found that will be most adaptable to man yet lethal to *C. immitis*.

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# Preliminary Observations on the Intravenous Use of Amphotericin B, an Antifungal Antibiotic, in the Therapy of Acute and Chronic Coccidioidal Osteomyelitis

M. L. Littman, M.D., Ph.D.

The greatest single public health need of the southwestern region of the United States is a clinically effective therapeutic agent for the treatment of disseminated coccidioidomycosis and its distressing, incapacitating, and often fatal complications. Until the present time there has been no effective specific medical therapy for this disease, although many medications have received extensive therapeutic trials.

Amphotericin B,* an antifungal antibiotic, was recovered from an unidentified streptomycete culture in 1956 by Gold, Stout, Pagano, and Donovick(1) and its isolation and characterization were accomplished by Vandeputte, Wachtel, and Stiller.(2) The former group found amphotericins A and B highly effective *in vitro* against a wide variety of human pathogenic fungi, and without effect against bacteria at concentration of 50μg./ml. Amphotericin B, the more active of the two compounds, was noted by Sternberg, *et al.*(3) to produce a protective and therapeutic effect in mice in a daily dosage ranging from 10 to 25 mg./kg. when it was administered intraperitoneally 24 hours after intraperitoneal infection of the mice with *Coccidioides immitis*. The $LD_{50}$ of a suspension of amphotericin B in mice was 280 mg./kg. when given intraperitoneally.(4) Although acute intravenous toxicity for mice was negligible at 15 mg./kg., doses of 20 mg./kg. and higher resulted in immediate toxicity with death. The chronic toxicity of the drug administered intraperitoneally was low, in that mice tolerated 5 to 100 mg./kg. daily for approximately 2 months without toxic effect.(3)

This report concerns the oral and intravenous use of amphotericin B in two patients with coccidioidal osteomyelitis. Although the clinical results with the intravenous form of the antibiotic described below are favorable, they are only preliminary in nature. The true value of intravenous amphotericin B in these and other cases of coccidioidomycosis can be evaluated only after a sufficient period of time has elapsed to permit adequate clinical and roentgenographic examinations, so that relapses, if any, may be observed and recorded. The evaluation of specific medical therapeutic agents for the chronic deep mycoses like that for tuberculosis is long term in nature.(5) Since this paper involves one of the first clinical uses of intravenous amphotericin B in the therapy of coccidioidomycosis, it was thought to be sufficiently important and timely to warrant presentation at this conference. A detailed report with more adequate follow-up will be published at a later date.

## Methods

### Assay of amphotericin B in body fluids

**Test culture and preparation of inoculum**: A standard strain of *Candida albicans*, Squibb No. 1539, was cultivated on yeast extract agar** slant for 48 hours at 37° C. A smooth suspension of this culture was prepared in sterile physiological saline to measure 50 percent light transmission with a photoelectric colorimeter (Lumetron Model 400-A), employing a red filter (650 mμ.). Stock cultures of the organism were maintained in sterile skim milk in a freezer at -20° C.

**Assay culture broth**:

| | |
|---|---|
| Penassay Medium, dehydrated, Difco | 13.2 g. |
| Dextrose, C. P. | 2.5 g. |
| Distilled water | 250 ml. |

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*E. R. Squibb & Sons, New York. Trade names are used for identification only and their use does not constitute endorsement of the product by the Public Health Service.

**Powdered yeast extract 0.3 percent; peptone 0.5 percent; dextrose 1 percent; agar 2 percent; pH 5-6.

The ingredients were dissolved in distilled water in a 500 ml. Erlenmeyer flask, autoclaved at 121° C. (15 lbs.) for 15 minutes and cooled, after which 10,000 units of penicillin and 25,000 units of streptomycin were aseptically added to provide concentrations of 40 and 100 µg./ml., respectively (20 and 50 µg./ml., respectively, in the final test). The stock liquid assay medium was mixed with the antibiotics and stored in the refrigerator at 5° C. Five-tenths ml. amounts of assay culture medium were aseptically dispensed into sterile Wassermann tubes on the day of the determination. Six tubes were required for each serum amphotericin B assay (table 1).

**Test procedure**: Body fluids under test were added in decreasing quantities to tubes containing 0.5 ml. of assay culture medium, and the final volume in each tube was made up to 1 ml. with sterile physiological saline (table 1). The inoculum consisted of one drop of saline suspension of *C. albicans* cells, as prepared above, delivered with a sterile 1 ml. serologic pipette to the fluid in each of the six numbered tubes. The drop of inoculum contained approximately 1 x $10^5$ yeast cells and did not impart visible turbidity to the culture medium. Tubes were incubated for 24 hours at 37° C., then shaken and examined visually with ordinary illumination for the presence or absence of turbidity (growth). Tubes with fungistatic dilutions of test fluid were clear after 24 hours of incubation, while others were turbid (fig. 1). In the initial examination of serum assays, it was found helpful to look first for the presence or absence of circular "buttons" of sedimented yeast growth in the bottom of the unshaken tubes to verify the fungistatic action of the test fluid. Blood serum obtained before the start of amphotericin B infusion was assayed simultaneously with the test serum.

In order to compute amphotericin B level of the test fluid using the above technic, it was necessary to first ascertain the minimal inhibitory concentration (MIC) of amphotericin B towards *C. albicans*, Squibb No. 1539, under approximately the same conditions as the serum assay. This was performed in single strength Penassay Broth containing 1 percent dextrose, to which was added 10 percent sterile serum (horse, rabbit, or human). Sufficient quantities of amphotericin B solutions (100 and 1000 µg./ml.), dissolved in 100 percent dimethyl sulfoxide, were aseptically added to 10 ml. quantities of this enriched culture medium to provide dilutions of the antibiotic ranging from 0 to 3.0 µg./ml. Upon thorough mixing of the antibiotic culture broth, 1 ml. of each dilution was aseptically transferred to sterile Wassermann tubes. Each 1 ml. then received one drop of *C. albicans* suspension as prepared above, delivered with a sterile 1 ml. serologic pipette. Tubes were incubated at 37° C. for 24 hours and examined for the presence or absence of turbidity. MIC values for *C. albicans*, Squibb No. 1539, performed by this method, averaged 0.09 µg./ml. and were rechecked once weekly. Stock amphotericin B solutions dissolved in dimethyl sulfoxide were stored in the freezer at -20° C. and assayed periodically for deterioration.

Body fluids were assayed unfiltered since both suspension and soluble forms of amphotericin B are retained by pyrex glass bacterial filters. Because normal urine may exert a growth inhibitory effect on *C. albicans*, depending upon its concentration of urea, bioassay of urine for amphotericin B is inaccurate. The assay of hemolyzed blood sera was avoided. Unhemolyzed specimens were assayed on the day they were collected.

TABLE 1

TECHNIC OF ASSAY OF AMPHOTERICIN B IN BODY FLUIDS

| Ingredient | Tube number | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Sterile stock assay medium, ml. | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Blood serum, urine, or spinal fluid, ml. | 0.5 | 0.3 | 0.2 | 0.1 | 0.05 | 0 |
| Sterile physiological saline, ml. | 0 | 0.2 | 0.3 | 0.4 | 0.45 | 0.5 |
| Suspension of *C. albicans*, drop | 1 | 1 | 1 | 1 | 1 | 1 |

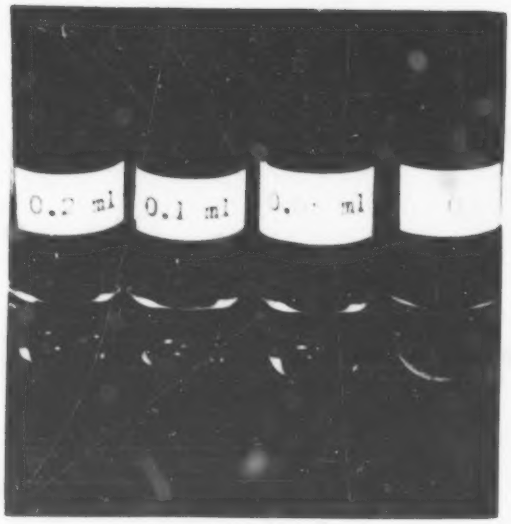


Figure 1. Serum amphotericin B assay, after 24 hours incubation at 37° C. Tubes with patient's serum are clear, indicating a fungistatic effect on the assay culture, *C. albicans*, Squibb No. 1539. The control tube without test serum is turbid.

**Calculations:**

$$\text{Amphotericin B level in test fluid, } \mu\text{g./ml.} = \frac{1}{\text{Smallest volume of test fluid showing a fungistatic effect.}} \times \text{MIC of amphotericin B on } C.\ albicans\ \#1539\ \mu\text{g./ml.}$$

When the smallest test volume in the assay is 0.05 ml. (table 1) with an MIC value of *C. albicans* at 0.09 µg./ml., the highest amphotericin B level which this test would detect is 1.8 µg./ml. The number of tubes in the assay can be increased to include smaller volumes of test fluid for estimation of higher serum levels.

***In vitro* sensitivity of *Coccidioides immitis*:** The growth of a mixed mycelial and arthrospore suspension of *C. immitis* isolated from Case I was inhibited *in vitro* after 4 days incubation at 37° C. by 0.5 µg./ml. of amphotericin B in Penassay dextrose broth enriched with 10 percent sterile horse serum.

## Therapeutic Results

### *Case I*

A 29-year-old white male chemist entered the Mount Sinai Hospital, New York, for the first time August 27, 1956, with complaints of an enlarged, tender, draining right knee of approximately 17 years duration.

History revealed that in 1930, at the age of three, he developed right mastoiditis, following which he was hospitalized for a widely disseminated staphylococcal osteomyelitis. In the ensuing 6 years of hospitalization, which was in the pre-chemotherapeutic era, his treatment consisted of incision, drainage, and immobilization of the affected extremities. He was discharged from the hospital at the age of 9 at which time the infection was arrested. For the next two years he resided continuously in Houston, Texas. In 1938, at the age of 11, he was sent for his health to Scottsdale, Arizona, a dusty, dry area in the Southwest, highly endemic for coccidioidomycosis. During his one-year residence in Scottsdale, he made frequent horseback trips into the desert where he was probably exposed many times to *C. immitis* contaminated dust. He had no bouts of fever, respiratory infections, arthralgia, or skin rashes during this period, however. In 1939, three months after moving to California, he developed an abscess of the right knee which, in the light of later developments, presumably was a metastasized coccidioidal lesion. Because of the mistaken impression that this represented a recrudescence of previously existing staphylococcal osteomyelitis, he was returned to the East where he was hospitalized for a 15-month period. The lesion was incised and drained, and finally healed. From this time until the present, the patient did not return to any endemic areas of coccidioidomycosis.

Following a symptom-free period of 8 years (1940-48), the patient, then 21 years of age, again developed pain and edema of the right knee, from which as much as 200 ml. of cloudy, yellow, synovial fluid was aspirated at monthly intervals from 1950 to 1952. A diagnosis of chronic synovitis was made, and an arthrotomy of the knee joint was performed in June 1952. At operation, the undersurface of the patella appeared softened and was shaved away. Intra-articular adhesions were also excised and a loose medial cartilage was removed. The synovial membrane was found to be hypertrophic and erythematous. Pathological examination of this membrane revealed edema, infiltration with chronic inflammatory cells, and a few tubercles containing foreign body giant cells, but neither bacterial nor fungal parasites were observed. A flexion contraction of the leg occurred which was corrected, following which a draining sinus of the knee appeared. In the following year, the patient fell on his right knee and his extremity was immobilized with a plaster cast for one month. His knee continued to give pain and discomfort until 1956 when, at a second operation, a granular mass was removed from the right popliteal region, after which a draining sinus developed. A diagnosis of tuberculosis of the right knee was made at that time, although without a positive culture for *Mycobacterium tuberculosis* or a biopsy for acid-fast bacilli. The patient received a course of streptomycin without effect. A diagnosis of coccidioidomycosis was next entertained but a coccidioidin skin test was negative and the laboratory failed to isolate *C. immitis* from the draining sinus. Amputation of the extremity was recommended by several consultant surgeons. In July 1956, a third operation was performed on the knee and an abscess involving the entire upper osseous cavity of the right tibia was discovered. The abscess was filled with soft, pliable tissue but no pus, and it communicated posteriorly with the sinus in the popliteal fossa. Profuse bleeding occurred during removal of the tissue.

It was at this point that I first saw the patient and examined the pathological material and stained tissue sections. A few *C. immitis* spherules without endospores were found in the stained tissue sections. Culture of the excised bone marrow and abscess material at 37° C. for 60 hours on liver spleen glucose blood agar(6) yielded many colonies of *C. immitis*. Bacterial cultures yielded an antibiotic-resistant, hemolytic *Micrococcus pyogenes* var. *aureus*. Skin tests with coccidioidin 1-100 were 2+; histoplasmin 1-1000, 3+; blastomycin 1-1000, –; *Cryptococcus neoformans* antigen 1-1000, –; and tuberculin first strength (Purified Protein Derivative), 1+. Serological studies revealed a positive complement fixation titer for coccidioidomycosis of 1:32 and a negative precipitin titer. A diagnosis of chronic coccidioidal osteomyelitis and chronic staphylococcal osteomyelitis was made and the patient was admitted to the hospital for treatment with amphotericin B.

Physical examination revealed a 126-lb., chronically ill, white male, who appeared 10 years older than his chronological age and who walked with the aid of crutches. He had many deep, healed osteomyelitis scars on the left shoulder, orbital region, ankle, right knee, and right foot, as well as a flexion contraction of the right arm. Other physical findings were right deafness, a moderate systolic and diastolic hypertension (BP 150/100), enlarged right deep inguinal lymph nodes and an enlarged tender right knee. On the anteromedial aspect of the upper half of the right leg there was a healed scar measuring 15 cm. long, the upper half of which contained a draining sinus bordered with numerous excrescences of reddened, firm, granulation tissue from which exuded a copious serosanguineous fluid (fig. 2). A second smaller draining sinus, 2 cm. in diameter, was also present in the right popliteal fossa. Range of motion of the right extremity was limited to an arc of 90° to 175°. Heart, lungs, and abdomen were normal.

Laboratory studies revealed hemoglobin 12.5 grams; red blood cell count 4,200,000/cu.mm.; white blood cell count 8600/cu.mm.; differential cell count: polymorphonuclears 57 percent; band cells 5 percent; lymphocytes 28 percent; monocytes 7 percent; eosinophiles 3 percent; blood platelets 282,000/cu.mm.; blood sedimentation rate 112 mm./hr. (Westergren); blood urea nitrogen 15 mg. percent; creatinine nitrogen 2.9 mg. percent; blood sugar 103 mg. percent; total serum protein 6.1 grams percent; serum albumin 2.8 grams percent; serum globulin 3.3 grams percent; and a 2-plus albuminuria. Serum electrophoresis revealed a shift in globulin distribution with a preponderance of $alpha_2$ and beta globulins and lowered gamma globulin.

**Course in hospital**: Roentgenograms revealed an extensive destructive lesion involving the right tibial metaphysis, diaphysis, and epiphysis, as well as adjacent subperiosteal bone (fig. 3). There was little surrounding bone reaction. The destructive process was a panosteitis, involving the medullary cavity, cortex, and periosteum. With the knee joint under fluoroscopic observation, iodochlorol (Searle) was slowly injected into the draining mediolateral sinus tract and roentgenograms were taken. Examination of these films revealed the presence of intercommunicating sinuses extending from the point of instillation posteriorly to the popliteal sinus tract and thence superiorly along the posterior surface of the femur for a distance of approximately 8 cm. (fig. 4).

A complete bone survey, including chest X-rays, failed to reveal additional lesions. Cultures of blood, urine, sputum, and sternal bone marrow were negative for *C. immitis*. Histological examination

Figure 2. Beneficial effect of intravenous amphotericin B on chronic coccidioidal osteomyelitis of the knee. RIGHT: The draining sinuses healed completely, coincident with the use of this antibiotic.

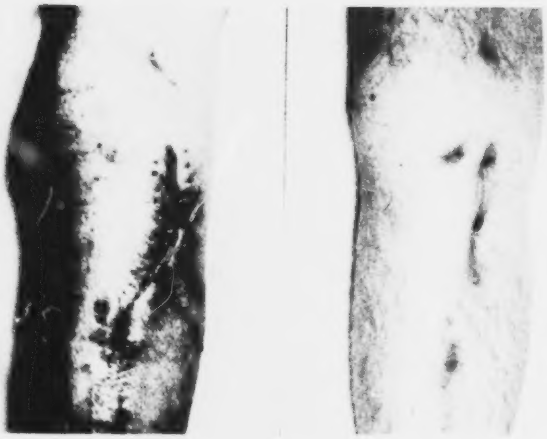


Figure 3. Osteolytic coccidioidal lesion of the right tibia, involving medullary cavity, cortex, and subperiosteum. There is little surrounding bone reaction. Normal left knee for comparison.

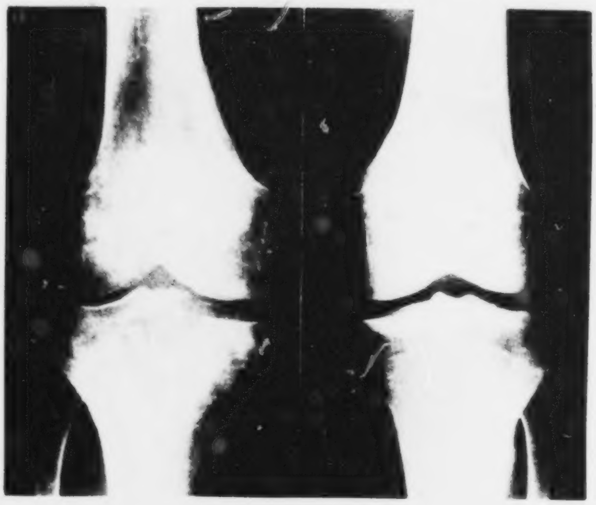
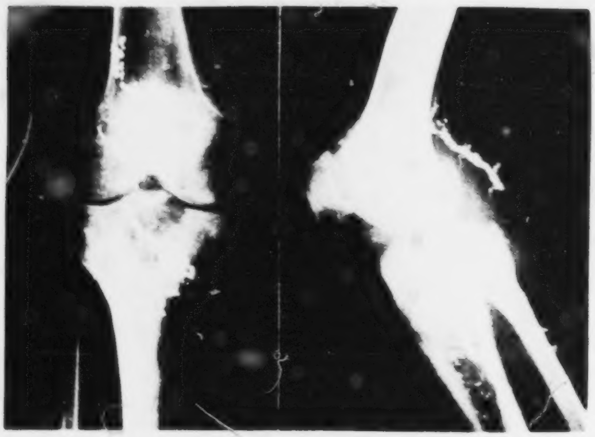


Figure 4. Iodochlorol-injection of the mediolateral sinus tract (see fig. 2) revealing presence of intercommunicating sinuses extending to the popliteal region and superiorly along the posterior surface of the femur.



of sternal bone marrow was negative, except for an increase in eosinophilic cells. A renal biopsy revealed extensive amyloid deposition, focal areas of tubular damage with cloudy swelling, hyaline droplet changes, and epithelial regeneration, all of which were attributed to the chronicity of the osteomyelitis. Electrocardiogram revealed only minor changes.

**Oral administration of amphotericin B:** Oral tablets of amphotericin B (Lot No. 691-11/15-A-5) were administered in divided doses in amounts increasing gradually from 3 grams per day until a daily dose of 7.2 grams was reached at the fourteenth day of treatment. This dosage was maintained for 24 more days without the occurrence of side effects such as nausea, vomiting, or other signs of gastrointestinal irritation, until a total of 226 grams had been administered. Significant changes in blood chemistry did not occur during this time. The patient received, in addition, daily infusions of 15 to 30 million units of penicillin for treatment of the coexisting staphylococcal osteomyelitis. He made no significant progress, however, during oral amphotericin B therapy. In fact, there was an increase in pain, tenderness, and edema of the knee during therapy; and no change was noted in the appearance of the draining sinuses. During the period of oral therapy numerous serum amphotericin B assays failed to reveal any of the drug in the blood serum. A single oral dose of a mixture of 2 grams amphotericins A and B (Lot No. 691-11/15-J) failed to produce any fungistatic or fungicidal effect in the patient's blood serum drawn 1¼, 3¼, and 5½ hours after administration. No side effects of the medication were encountered. It was concluded from these observations that oral preparations of amphotericins A and B, in their present form, were too poorly absorbed from the gastrointestinal tract to be clinically and systemically effective and they were discontinued.

It is of interest to note that Fiese(7) obtained a beneficial effect with oral amphotericin B in a 27-year-old white male with generalized coccidioidomycosis. This patient had received 2.4 grams of the drug daily by the oral route for a period of three months, for a total of 200 grams. Blood serum levels were not reported.

**Intravenous therapy with amphotericin B:** On October 7, 1956, a suspension of amphotericin B for intravenous use (Lot No. 691-14/15-4-F) was made available and it was administered to the patient. Coincident with the start of intravenous amphotericin B therapy and during the ensuing four month period of treatment, the patient made steadily progressive improvement. Tenderness, pain, and edema of the knee subsided shortly after the start of this treatment, and complete healing of the lesions eventually followed (fig. 2). The first intravenous dose consisted of 50 milligrams of amphotericin B (0.8 mg./kg.), suspended in 500 ml. 5 percent glucose in distilled water, and administered slowly to the patient over an 8-hour period. Since normal saline is not recommended by the manufacturer as a diluent for intravenous amphotericin B, it was not employed. The first infusion was well tolerated. The daily dose of amphotericin B was gradually increased by suspending increasing quantities of the antibiotic in 500 ml. 5 percent glucose, until at the close of a three-week period, it had reached 150 mg. (2.6 mg./kg.). This dose was administered daily for 4 days, after which the patient's blood urea nitrogen rose to 50 mg. percent from a normal of 15 which necessitated discontinuance of the antibiotic. After a three-week-rest period, during which time the blood urea nitrogen level had returned to normal, the patient received amphotericin B again in smaller doses, not exceeding 100 mg. daily (1.6 mg./kg.). Azotemia did not occur when the antibiotic was given intravenously at this dose on alternate days, and 33 more intravenous doses were administered without this complication. Cultures of the sinus tracts became negative for *C. immitis* shortly after the start of intravenous therapy. Side effects throughout the entire period of infusion therapy were not serious. They occurred during the infusion and consisted of chilliness, febrile reactions which could be controlled fairly effectively with salicylates and less so with antihistaminic drugs, nausea, vomiting, flushing, perspiration, fatigue, and drowsiness; but all of them were temporary in nature and subsided promptly upon discontinuance of the medication. On several occasions, transitory episodes of vestibular disturbances were encountered.

During the months of December 1956 and January 1957, while the patient was receiving 100 mg. amphotericin B in 500 ml. 5 percent glucose in distilled water on alternate days, there were no significant alterations in hemoglobin, white blood cell count, blood urea nitrogen, or liver function tests. The incidence of drug-induced thrombophlebitis with amphotericin B was no greater than that of other infusion medications such as penicillin. A total of 56 intravenous infusions of amphotericin B were administered over a period of 118 days during which 4700 milligrams of the antibiotic were administered.

**Blood serum amphotericin B levels**: Doses of 25 and 50 milligrams amphotericin B (Lot No. 691-14/15-4-G) (0.4–0.8 mg./kg.), suspended in 500 ml. 5 percent glucose in distilled water, administered intravenously over a 7-hour period, failed to produce a measurable blood serum level by the aforementioned assay technic (table 1). Doses of 100 and 140 milligrams (1.6-2.4 mg./kg.) given in the same manner, however, produced blood serum levels of 0.9 and 1.8 µg./ml., respectively. The persistence of amphotericin B levels in the blood serum 12 and 18 hours after the antibiotic had been discontinued (table 2) indicated that conjugation of the antibiotic by the liver was apparently not very great.

Additional therapy consisted of (1) immobilization of the extremity with a half-spica plaster cast, with a window over the lesion; (2) intravenous penicillin in doses varying from 15 to 30 million units daily for treatment of the coexisting staphylococcal osteomyelitis; (3) injections of autogenous staphylococcal, heat-killed vaccine; (4) frequent injections of vitamin B complex. The penicillin sensitivity of the *M. pyogenes* var. *aureus* increased from an initial level of 0.5 units per ml. in September 1956 to 2.0 units per ml. in January 1957. During one infusion of 15 million units of penicillin in 500 ml. 5 percent glucose in distilled water, the patient's blood serum assayed 33 units of penicillin per ml.

Serological studies at the end of therapy revealed a definite fall in complement fixation titer for coccidioidomycosis to 1:16, associated with the patient's clinical improvement, and a negative precipitin titer. Skin tests at this time with coccidioidin 1-100 were 1+; histoplasmin 1-1000, 2+; blastomycin 1-100, –; *C. neoformans* antigen 1-1000, –; tuberculin first strength (Purified Protein Derivative), 1+.

Roentgenograms of the right knee in January 1957 revealed a distinct filling-in with bone in the areas of osteolytic destruction of the tibia and the subsidence of subperiosteal reaction. The patient was fitted with a brace to assist in weight-bearing and discharged from the hospital with a healed knee on February 15, 1957, to return at periodic intervals for evaluation and follow-up.

TABLE 2

ASSAYABLE LEVELS OF AMPHOTERICIN B IN BLOOD SERUM COINCIDENT WITH INTRAVENOUS THERAPY (CASE I)

| Dose of amphotericin B suspension* in 500 ml. 5% glucose in distilled water | | Duration of infusion, hrs. | Time specimen obtained | Amphotericin B level, in blood serum, µg./ml. |
|---|---|---|---|---|
| mg. | mg./kg. | | | |
| 25 | 0.4 | 7 | During infusion | 0 |
| 50 | 0.8 | 7 | During infusion | 0 |
| 100 | 1.6 | - | Before infusion | 0 |
| | | 7 | During infusion | 0.9 |
| | | - | 12 hrs. after discontinuance | 0.9 |
| | | - | 18 hrs. after discontinuance | 0.9 |
| | | - | 38 hrs. after discontinuance | 0 |
| 140 | 2.4 | 8 | During infusion | 1.8 |

*Lot No. 691-14/15-4-G (Suspension).

### *Case II*[*]

A 23-year-old Negro male was admitted to the Veterans Administration Hospital, Bronx, N. Y., for the first time December 27, 1956, with complaints of swelling and pain of the left knee and extremity, of approximately three years duration.

History revealed that he had lived in Mississippi until the age of 10 years, at which time, in 1944, he moved to Los Angeles, California. Before his induction into the U. S. Army in 1951, he had traveled frequently to Bakersfield, California, one of the Southwest's most highly endemic areas of coccidioidomycosis. He could recall no episodes of fever, respiratory infections, skin rashes, or arthralgias during this time. In 1951, while stationed in Korea, he fell from a military vehicle and injured his left knee, for which he was hospitalized and treated symptomatically. In 1955, he again injured the same knee. In 1935, he developed swelling and tenderness of the left knee, which was treated by aspiration and intra-articular injection of hydrocortisone with a consequent reduction of swelling and pain. When the patient was discharged from the service in June 1956, however, he still had an enlarged, stiff, and painful left knee. In September 1956, he was involved in an auto accident and again injured the same knee, which then became grossly enlarged. A chain of symptoms began, including progressive weight loss of 20 pounds, frequent night sweats, 4+ pitting edema of the left lower extremity, and the appearance of systolic and diastolic heart murmurs; and he was admitted to the hospital in December 1956, with a diagnosis of subacute bacterial endocarditis and traumatic arthritis.

Physical examination revealed an acutely ill, 140-pound, feverish, Negro male, with a swollen left thigh, edematous lower extremity, and a grossly enlarged, tender, warm, left knee. The left extremity was held rigidly in flexion since movement in any direction caused the patient considerable pain. Other physical findings were a grade II, loud, harsh systolic apical murmur, grade III diastolic murmur, and enlarged left deep inguinal lymph nodes. The liver and spleen were not enlarged. Roentgenogram of the left knee revealed pericapsular swelling, synovitis, and minimal destructive changes consistent with either tuberculous disease or pyogenic osteomyelitis.

Laboratory studies revealed hemoglobin 11.8 grams; red blood cell count 4,000,000/cu.mm.; white blood cell count 7200/cu.mm.; differential cell count: polymorphonuclears 58 percent; lymphocytes 24 percent; monocytes 6 percent; and eosinophiles 12 percent; blood platelets 198,000/cu.mm.; sedimentation rate 70 mm./hr. (Westergren); blood urea nitrogen 6 mg. percent; total serum protein 7.7 grams percent; serum albumin 3.0 grams percent; serum globulin 4.7 grams percent; cephalin flocculation 2+; and thymol turbidity 5.7 units. Serum electrophoresis revealed an increase in alpha$_2$ and gamma globulin components. Electrocardiogram revealed elevation of ST segments in chest leads $V_3$ to $V_6$.

**Course in hospital:** On the third day in the hospital the patient became acutely febrile and unresponsive to large intravenous doses of penicillin and erythromycin. During the ensuing four weeks his temperature spiked daily to 104°-105°, despite medications. A series of 18 blood cultures taken during this period, as well as aspirated knee fluids, were negative for bacteria. Cultures for fungi, however, were not performed. A synovial biopsy on January 23, 1957, revealed numerous *C. immitis* spherules with endospores. Aspiration of the left suprapatellar bursa yielded 40 ml. of bloody, purulent fluid, from which *C. immitis* was recovered in large numbers on liver-spleen glucose blood agar.(6) Cultures of blood, urine, and sputum, were negative for this fungus. Serological studies revealed a high positive complement fixation titer for coccidioidomycosis of 1:256 and negative precipitin titer, compatible with extensive extrapulmonary disease. Skin tests with coccidioidin were not performed at this time because of the severity of the disease, and the fear of hypersensitivity reaction.

**Intravenous administration of amphotericin B:** The first dose of 50 milligrams of amphotericin B suspension (0.8 mg./kg.) in 500 ml. 5 percent glucose in distilled water was accidentally allowed to flow in too rapidly, i.e., 28 milligrams in 40 minutes, causing convulsions and ventricular fibrillation leading to a temporary cardiac arrest. This was fortunately counteracted by striking several sharp blows on the patient's chest. The next day a similar dose was given slowly over an 8-hour period and it was well tolerated. The third and succeeding doses, consisting of 50 milligrams of a newer, water soluble salt of amphotericin B (Lot No. 691-14/15-7) dissolved in 500 ml. 5 percent glucose in distilled water, were well tolerated when infused slowly over a 6-to-8-hour period. The dose was repeated on alternate days and rapidly increased to 75 milligrams daily (1.2 mg./kg.). Coincident with each infusion of soluble amphotericin B, the patient's temperature markedly dropped within 12 to 18 hours and his general condition improved. By the eighth infusion, he was afebrile and able to sit up comfortably in bed, after nearly one month of unremitting high fever and prostration. There was no rise in blood urea nitrogen during treatment on alternate days with amphotericin B. Intra-articular instillation into the left knee joint of 25 milligrams of soluble amphotericin B dissolved in 7.5 ml. sterile distilled water was well tolerated without any immediate or delayed discomfort or pain. Following this, a sharp reduction was noted in the number of colonies of *C. immitis* in joint fluid cultures. After the tenth intravenous infusion of amphotericin B, the patient's general condition improved sufficiently to enable him to undergo an extensive incision and drainage of the left knee. At operation, considerable pus was evacuated, devitalized tissues excised, the wound washed and packed with amphotericin B solution, and rubber drains inserted. The left extremity was immobilized with an open, half-spica plaster cast. After the operation, the patient continued to receive intravenously, on alternate days, 75 milligrams of amphotericin B in 500 ml. 5 percent glucose in distilled water. In addition, 25 milligrams of the antibiotic dissolved in distilled water, were injected intra-articularly three times weekly. At present, the patient is still under treatment, but is now afebrile and no longer acutely ill. It is planned to continue the same treatment for at least several months, after which he will be observed for an adequate period of time.

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## Summary

A beneficial clinical effect was observed in two patients with acute and chronic coccidioidal osteomyelitis, who were treated with intravenous amphotericin B, an antifungal antibiotic derived from a streptomycete. An oral preparation of this antibiotic had proved of no value in one patient. Adequate and sustained blood serum levels of amphotericin B, measurable by a bioassay technic were achieved by intravenous administration. The culture of *C. immitis* isolated from one of the patients was inhibited *in vitro* by 0.5 μg./ml. amphotericin B in 4 days at 37° C. in the presence of serum. A number of toxic side effects of amphotericin B, noted in both patients, were minimized by slow intravenous administration of the antibiotic on alternate days and by the administration of aspirin.

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# Immunological Studies on Coccidioidomycosis

Lorraine Friedman, Ph.D.

Immunity in coccidioidomycosis can be divided into two categories: that which is passively acquired and that which is actively acquired. Neither type has been investigated extensively, but both offer defenses against this disease. Passively acquired immunity suggests the possibility of treatment and could be used to study the function of serologically detectable antibodies. Actively acquired immunity introduces the possibility of preventing coccidioidal disease. I should like to present our experimental approaches to immunity, the reasons for such approaches, and some of the information thus far obtained in our efforts to develop a vaccine.[1]

When considering artificial active immunization against this disease, the knowledge we have concerning naturally acquired immunity is most encouraging. As far as we now know even a subclinical infection with *Coccidioides immitis* leaves the individual relatively resistant to a second infection. Also, the presence of some type of natural immunity is indicated by the fact that individuals with cavitations rarely disseminate their infections from these cavities despite the presence of normal, viable organisms that persist for long periods of time. This evidence attesting to the strength of naturally acquired immunity gives hope to the possibility of inducing immunity artificially through vaccination.

Previous attempts by Dr. C. E. Smith (unpublished) to immunize with dead cells and by Negroni and his associates in South America[2] to elicit immune response with the mycelial phase were not encouraging. Some felt the explanation was the occurrence of the fungus as spherules, rather than mycelia, in the disease process so that only a vaccine of spherules would be effective. Therefore experimentation by others was probably postponed awaiting development of methods to culture the fungus in the form of spherules.

Inoculation with a living, attenuated strain, such as that used against tuberculosis, might have seemed worth considering except for our survey of *C. immitis* strains.[3] The survey showed that only a few strains were of low virulence for laboratory animals, none were avirulent, and the least virulent strain has been known to increase unexpectedly in virulence. Another possibility was that of immunizing with a related organism. Unpublished work, in Smith's laboratory, had shown us that mice inoculated with viable *Histoplasma capsulatum* were resistant to infection with *C. immitis* and that even killed cells seemed to offer some protection. At the same time we were reminded that in 1927 Hirsch and D'Andrea[4] were able to sensitize guinea pigs with killed mycelia. This indicated to us that this form of the fungus was not immunologically inert.

Dr. Smith and I felt that the two forms of the fungus were not too distantly related immunologically, for individuals with coccidioidomycosis, and hence spherules, responded when skin-tested with coccidioidin, a broth filtrate of the mycelial phase. Therefore, we decided to re-explore immunization with killed cells of the mycelial phase of *C. immitis*.

Our first vaccine consisted of saline-suspended arthrospores of the Silveira strain, which produces characteristic morphologic forms when grown on a solid medium. In general, the procedure with this type of preparation has been to culture the fungus at 34° C. and collect the spores when the agar dried. At that time this strain consists almost entirely of spores with very few hyphae. The resultant powdery material is exposed to formaldehyde gas until sterile. This can be accomplished in about 48 hours at room temperature, and faster at 37° C. when the container of spores is placed in a closed chamber with formalin in the bottom. After removing the material from the chamber, storage under atmospheric conditions, preferably with some aeration, quickly frees the spores of formaldehyde as detectable by odor. The material can then be suspended in saline by shaking, and the percentage by volume can be determined by centrifugation in a hematocrit tube, much the same way as the packed cell volume of red blood cells is determined.

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Because handling dry spores is hazardous, a second method of vaccine preparation has been tried. The fungus was grown in a constantly agitated liquid medium, as described by Roessler and his associates.(5) This method has necessitated the use of another strain, Cash, because the Silveira strain yields primarily mycelia rather than spores. It was also thought that sterilization with acetone and grinding at 70° C. might yield a more antigenic preparation. Accordingly, both procedures have been incorporated. Nearly all of the acetone is removed by evaporation at 4° C., and the "moist" sediment is resuspended to approximately the same concentration as the dry spore vaccine.

In the choice of an experimental host we again considered earlier efforts. Both Negroni and Smith had used guinea pigs. However, recently Dr. Smith found these animals respond inconsistently to infection with *C. immitis*. Studies on pathogenicity reveal mice to have a relatively uniform susceptibility. Therefore, this animal was selected for the evaluation of our vaccines. Our experiments differ in yet another way from those of previous investigators. Knowing that fungi are frequently antigenically weak, we employed large inocula, 0.1 ml. of our suspensions adjusted to 20 percent by volume. For the results to be described, each vaccine was inoculated subcutaneously into groups of about 25 mice at approximately 5, 4, 3½, and 2 months prior to intraperitoneal challenge with a lethal dose (100 viable spores) of the Silveira strain.

Both types of vaccines conferred some protection, but neither was outstanding. There was one death among the mice inoculated with the formaldehyde-killed, dry-spore vaccine; and there were no deaths among those receiving the acetone-killed, submerged-culture vaccine. By comparison, half of the 100 non-vaccinated, control mice were dead in 19 days, and 80 percent were dead at the end of the observation period of 60 days.

Although the course of the disease was favorably modified in the vaccinated animals, the infectivity of *C. immitis* was only slightly reduced. At autopsy, 60 days after challenge, only 3 (10 percent) of the survivors which received the acetone-killed, submerged-culture vaccine and only 4 (13 percent) of those inoculated with the dry-spore vaccine appeared normal and gave negative cultures.

The latter findings were disappointing. However, complete protection of experimental animals against infection, although desirable, may prove too rigid a criterion of immunization. An immunologic response such as that engendered in these experimental animals might be sufficient to offer some protection to that percentage of the population destined under natural conditions to disseminate their infections. But if dissemination is a function of dose, as well as race, the prevention of this sequence might require an unusually strong immunity. It does not seem likely that infection of many individuals is initiated with doses proportionally as great as those with which these animals were challenged. Experiments are in progress to evaluate the protection conferred against challenges as high as 100,000 virulent spores. Also, the observation period has been extended to determine whether or not vaccinated mice would overcome their infections in longer periods of time.

Many questions must be answered. Is artificially acquired immunity transient or of long duration? Would the immunologic response be equally protective against a more natural challenge such as the respiratory route? Now that spherules appear to be readily obtainable in culture,(6) would a vaccine prepared from such material prove more protective?

It is worthwhile noting that Vogel *et al.*(7) sensitized guinea pigs with spherules cultured in eggs. These animals showed some suggestion of increased resistance when sacrificed a few weeks after challenge as compared grossly and microscopically with non-vaccinated animals.

Although much remains to be done, the results have established one encouraging fact, immunization, at least to some extent, against *C. immitis* is possible.

### Summary

An attempt has been made to outline a variety of experimental approaches to the development of a vaccine for coccidioidomycosis and to describe briefly the protection of mice against *C. immitis* infections by means of artificially induced, active immunity.

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## *Section III*

# Veterinary Aspects In Coccidioidomycosis

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# Nonfatal Coccidioidomycosis of Dogs

Raymond E. Reed, D.V.M.

Recognition that the dog is susceptible to natural *Coccidioides immitis* infection dates from 1940.(1) The disease has been reported in necropsy findings at intervals from that date to the present.(2-9) In 1955, Burger and Levan(10) reported the first confirmed canine coccidioidal infection with recovery.

In October 1952, a study of canine coccidioidomycosis was initiated in cooperation with practicing veterinarians in Tucson, Arizona. Clinical histories of dogs in which the disease was confirmed at necropsy were compared and a symptomatic picture compiled. When it became possible to recognize the disease with some accuracy during its clinical course, dogs tentatively diagnosed as suffering from coccidioidomycosis were subjected to coccidioidin sensitivity and serological tests.

It soon became evident that ante-mortem diagnoses could be made frequently enough to provide support of the clinical picture in numbers.(11) The clinical manifestations of canine coccidioidomycosis were associated with necropsy findings of dissemination in all but one case, diagnosis in the latter being based on high titer complement fixation.

Continued study of infections in dogs in Tucson, together with the findings of Maddy(12) in Phoenix, indicates that almost all dogs with a confirmed diagnosis of coccidioidomycosis are destroyed because of progressive disease. Death occurs without human intervention in a few instances, but the animal's chronic general debilitation usually leads the owner to request euthanasia.

## Methods

Presumptive diagnosis of coccidioidomycosis in the dog can be made on the basis of clinical findings (cough, persistent fever, loss of weight, fair to normal appetite, sporadic diarrhea, dull coat, hyperesthesia, or lameness) and the case history supported by radiographically demonstrable thoracic or skeletal lesions. A tentative diagnosis is further supported by failure of the infection to respond to antibiotics or chemotherapeutic agents.

Confirmation of the diagnosis in the living animal can be accomplished by culturing the fungus from respiratory exudate, gastric washings, feces, and exudates from cutaneous lesions. Culture or histopathologic examination of sections of biopsy specimens (lung, liver, bone, cutaneous lesions) may prove the diagnosis. Two or more serological tests(13) performed at intervals of two or three weeks may provide conclusive evidence. Sensitivity to intracutaneous inoculation of undiluted coccidioidin, if demonstrated after an initially negative test, may help confirm diagnosis,(14, 15) but there is seldom opportunity to observe dogs convert to the coccidioidin test. Although some dogs with the severely disseminated disease react poorly or not at all to coccidioidin, most of them respond with an area of induration greater than 10 mm. diameter. In a sample of 336 dogs tested, the correlation between certainty of diagnosis and degree of reaction yielded a coefficient of 0.855.

Besides physical signs of recovery, there is some evidence that, as in man, a drop in complement fixation titer may signal conquest of the disease. In an equally vague fashion, increase in coccidioidin sensitivity has been associated with recovery.

As information on individual, naturally infected dogs became part of the record, it was separated into four groups on the basis of clinical history, laboratory, and necropsy findings. Dogs with no history or with no symptoms suggesting coccidioidomycosis were classed as "normal." Sick dogs demonstrating some symptoms suggestive of coccidioidomycosis, but falling short of the characteristic pattern, were classed as "vague." If the symptomatic pattern was fulfilled, the dogs were classed as "typical cases"; and if diagnosis was definitely established by laboratory procedures or necropsy, they were classed as "confirmed." The initial classification was changed to another when new information so dictated.

As part of an experiment designed to establish incubation period, morbidity and mortality, suscep-

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tible dogs were infected by intratracheal insufflation with a suspension of arthrospores harvested from *C. immitis* cultures. This experiment will be in progress for some time, but preliminary results$^{(16)}$ are included in this report.

## Observations

Since the beginning of the study, 389 dogs have been under casual to intensive observation. Known survivals and deaths among naturally infected dogs are shown in table 1.

A few of the dogs in the "typical" group that lived less than 6 months after diagnosis was made, and even a few that have survived over 6 months, have disseminated coccidioidomycosis and certainly will die or will be destroyed. Fewer dogs appear in the "typical" category than in the "confirmed" because, as time passed, disseminations in the "typical" group led to destruction of the dogs, subsequent necropsy, and reclassification. Dissemination occurred in cases with mild to severe clinical symptoms as long as 18 months after apparent recovery. Some dogs that had the disseminated disease had no history of primary illness.

Very few dogs in the "vague" category were observed extensively, which explains the ignorance about the survivals. The fact that 184 of the 372 dogs included in this study could not be accounted for is in part due to inability to renew contact with the owners.

Dogs listed in the "normal" row were, for the most part, animals that were examined and tested for coccidioidin sensitivity as a means of reassuring their owners that the dogs were free of the disease. Usually the owners had lost another dog previously from the effects of disseminated coccidioidomycosis.

Results of artificial infection of young, susceptible dogs with *C. immitis* arthrospores are given in table 2. Obviously the dogs' chances of survival hinged on the number of spores administered. Surgical intervention to obtain biopsy specimens undoubtedly caused several premature deaths. Although one of the dogs infected with 10,000 viable *C. immitis* arthrospores and another infected with 1,000 spores were found to have disseminated disease at necropsy, both had shown evidence of recovering physically and had demonstrated a drop in complement fixation titer.

## Discussion

Opportunity to confirm diagnosis of coccidioidomycosis before necropsy was limited in this study of naturally infected dogs. With increased future use of serial complement fixation tests, more confirmed diagnoses will be made before the dogs are destroyed, but it is not anticipated that the proportion of survivors of the clinically recognizable disease will increase appreciably. Symptoms of coccidioidomycosis in the dog are sufficiently nonspecific to require prolonged observation before a diagnosis can be made. Performance of a complement fixation test does not usually occur to the observer until the diagnosis is fairly certain, and by that time the dogs may be close to, or have developed, a disseminating form of the disease. Survival of animals with disseminated disease would certainly continue to be the exception.

Recognition of natural primary coccidioidal infection in the dog will probably continue to be

TABLE 1

KNOWN DEATHS AND SURVIVALS AMONG TUCSON DOGS OBSERVED

| Diagnostic Category | Less than 6 mo. survival | Over 6 mo. survival | Known deaths | Outcome unknown | Total |
|---|---|---|---|---|---|
| Normal | 5 | 3 | 2 | 10 | 20 |
| Vague | | | 22 | 157 | 179 |
| Typical | 17 | 28 | 13 | 16 | 74 |
| Confirmed | 1 | 6 | 91 | 1 | 99 |
| Total | 23 | 37 | 128 | 184 | 372 |

## TABLE 2

### RESPONSE OF DOGS TO ARTIFICIAL INFECTION WITH *C. IMMITIS*

| No. dogs | No. viable arthrospores administered | Days survived | Cause of death | Extent disease progressed |
|---|---|---|---|---|
| 2 | 1,000,000 | 11, 13 | infection, thoracotomy | multiple focal pulmonary lesions, bronchial lymph node lesions |
| 2 | 1,000,000 | 31, 31 | euthanasia, 1 biopsied | same as above |
| 2 | 500,000 | 9, 10 | infection | same as above |
| 1 | 25,000 | 2 | not related to coccidioidomycosis | not established |
| 1 | 25,000 | 9 | infection, thoracotomy | same as first 6 above |
| 2 | 10,000 | 10, 33 | euthanasia, survived biopsy | same as above |
| 1 | 10,000 | >5 mo. | euthanasia | lesions in lung, bronchial lymph nodes, mediastinum and mediastinal lymph nodes, heart and pericardium, liver |
| 1 | 1,000 | >5 mo. | euthanasia | lesions in lung, bronchial lymph nodes, liver, spleen, kidney |
| 1 | 1,000 | >5 mo. | euthanasia | same as first 6 above but resolving |
| 1 | 1,000 | >6 mo. | living | biopsy proved coccidioidal lesions in lung but dog not destroyed |
| 3 | none | 31, 120, 120 | euthanasia | no infection |

almost impossible. Symptoms demonstrated by the artificially infected dogs varied in severity and in time of appearance in relation to number of spores inoculated, but in the range of mild to severe infections, symptoms were in no way specific for this disease. It seems impossible at this time to distinguish between the dog that is febrile, coughing, and showing mild inappetence and diarrhea as a result of coccidioidal infection, and his fellows that are showing the same response to infections due to bacterial or viral agents.

Of the dogs included in the study that were checked for sensitivity to intracutaneous injection of undiluted coccidioidin, 61.3 percent developed indurations of 5 mm. diameter or greater at the injection site. Dividing the dogs into groups according to the certainty of diagnosis, 50 percent of the "normal" group, 38.2 percent of the "vague" group, 98.7 percent of the "typical" group, and 94.7 percent of the "confirmed" group reacted with indurations of 5 mm. or greater in diameter in the injection site. In Phoenix, Maddy(12) found the distribution of dogs positive to coccidioidin to be 52.6 percent in normal dogs, 55.4 percent in dogs with some symptoms suggestive of coccidioidomycosis, and 81.8 percent in dogs in which the disease was proved. If coccidioidin sensitivity in the dog indicates tissue response to the fungus, a large portion of the dog population in the two cities convert without recognizable primary symptoms or present nonspecific symptoms. Most of the animals were treated in veterinary hospitals since they were owned by people accustomed to consulting a veterinarian when their pets became ill.

Morbidity and mortality rates can be established by observing the response of dogs to experimental infection with *C. immitis*, but the problem of early differential diagnosis will remain with the veterinary clinician. His knowledge of the disease in the dog, and his ability to recognize it, has been increased by this study in the two cities involved, but one of the most potentially useful results of this and Maddy's study is the suggested possibility of using the dog as an experimental animal for other phases of research in coccidioidomycosis. Histopathologically, the disease in the dog mimics that in man with one exception – canine bone lesions are proliferative while those occurring in man are destructive.

### Summary

The procedure for making and confirming a clinical diagnosis of canine coccidioidomycosis is briefly outlined. There are few survivals among naturally and experimentally infected dogs.

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# Coccidioidomycosis in Arizona Cattle

Charles J. Prchal, D.V.M.

Coccidioidomycosis is frequently found in cattle from an endemic area (1) at the time of examination of the thoracic viscera after slaughter. It is not an important disease to the cattle men and the meat packing industries because neither of them suffer monetary losses because of it. When the infection strikes in a herd of cattle, it goes unnoticed since it produces no visible ill effects. Lesions found at the time of slaughter are always localized in the lungs and in the immediate regional lymph nodes; therefore, condemnation of a part or all of a carcass is not necessary. The importance of coccidioidomycosis in cattle is limited to the fact that it is a reportable disease in this area.

The following observations have been consistent in this area for the past decade:

(1) The disease does not produce any respiratory disturbances or other symptoms in cattle that can be detected by a physical examination. Animals originating from known infected premises have been examined at times of rest and feeding; and when moved hurriedly from one pen to another, neither a disturbance in breathing nor a cough has ever been noticed. Infected animals have always been found to be in good flesh, alert, and without evidence of fever.

(2) Lesions of the disease have been found in cattle of all ages, in calves of 10 to 14 months of age, in steers and heifers in the two- to four-year class, and in cows and bulls five to nine years of age.

(3) Almost without exception the lesions are found in those cattle that have been in pen-feeding areas. If they have never been pen-fed or confined to close quarters, cattle of all ages that originate on open range pastures – whether desert or mountainous type – are found to be free of lesions when slaughtered.

(4) The number of animals of a given lot in which lesions have been found varies with the location of the pen-feeding areas from which they come. Some feeding lots have consistently furnished animals for slaughter that are free of lesions, while up to 20 percent of animals from other feeding lots have lesions.

(5) The lesions found have been limited to and localized in the lungs and the bronchial and mediastinal lymph nodes. We have never found multiple lesions in the lungs, lesions that have become disseminated, or any outside the thoracic cavity.

The macroscopic appearance of a lung lesion can be best described as a circumscribed, encapsulated area of purulent granulomas. Distinct granulomas appear in early infections, as shown in figure 1. The granulomas contain a central core of adhesive purulent material that is usually a light cream color. Surrounding the granuloma is pneumonic tissue which is customarily in the stage of red hepatization. A well organized connective-tissue capsule envelops the entire lesion.

Macroscopically, an infected lymph node appears to be swollen two to four times its normal size. When incised the exposed surfaces present multiple granulomas with purulent centers. Usually, the greater part of the lymph node is affected, and the unaffected part appears as densely packed lymphoid tissue. The infected node is surrounded by a well defined connective-tissue capsule.

Presumably, the variances seen at times in lesions in the lungs and the lymph nodes are the result of the defensive mechanisms of the infected animal. The granulomas are believed to exist, as such, for only a short time, after which they fuse together and the purulent material is held in one large area by connective tissue. A caseated or a caseo-calcareous mass may be formed prior to final absorption of the lesion.

Microscopic examinations of countless lesions were made by Dr. C. L. Davis (retired), former Chief, Federal Pathological Laboratory of the United States Department of Agriculture. A typical report of his findings reads "Sections of the lung and lymph node showed the lesions to be encap-

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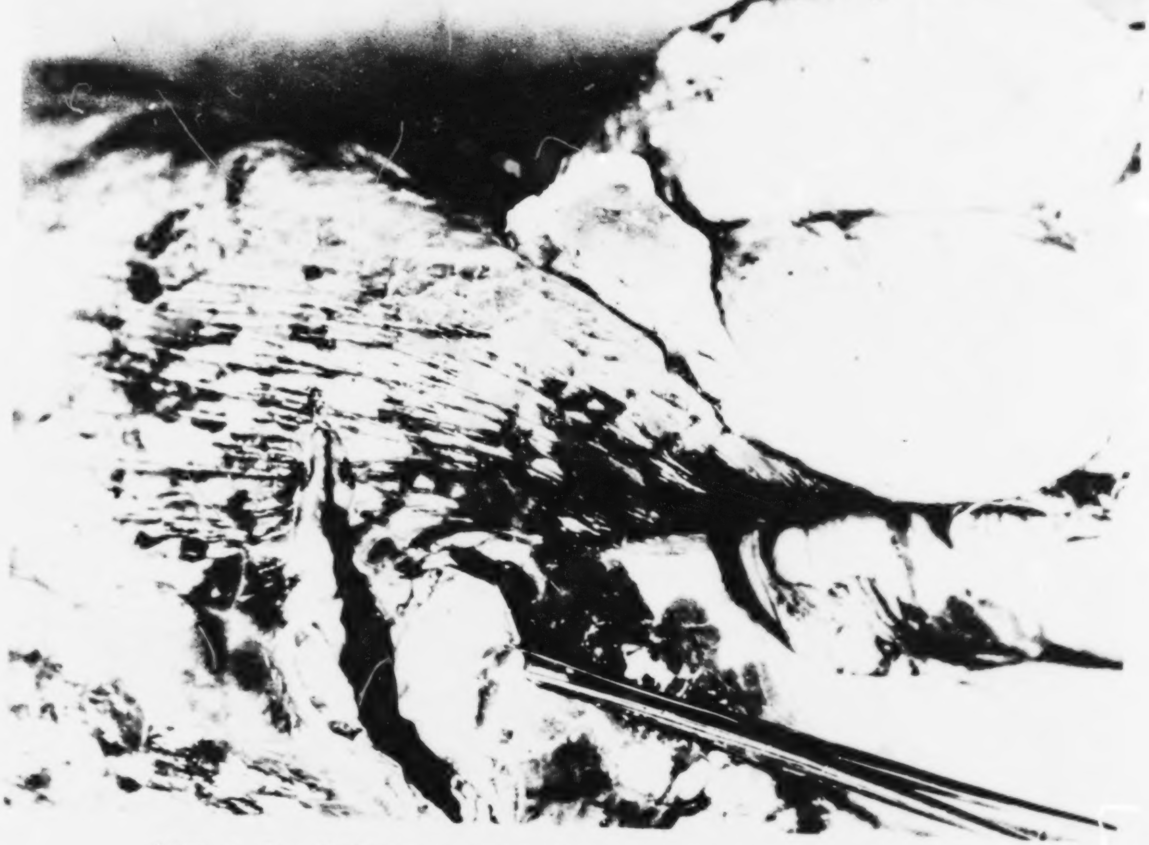

Figure 1. Coccidioidomycosis, bovine. Lung and mediastinal lymph node lesions.

sulated granulomas made up of epithelioid cells, giant cells and a heavy infiltration of inflammatory cells of the chronic type. Focal accumulations of polymorphonuclear leucocytes are also evident. Typical spherules of *Coccidioides immitis* were demonstrated, some within giant cells and others surrounded by pus cells."

## Summary

A brief description of coccidioidomycosis in Arizona cattle is presented with special emphasis on the type of lesions found at time of slaughter.

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# A Study of One Hundred Cases of Disseminated Coccidioidomycosis in the Dog

Keith T. Maddy, D.V.M., M.P.H.

Coccidioidomycosis has been considered to be an uncommon infection in animals. However, recent studies indicate that many of the wild and domestic mammals, especially dogs, that live in the coccidioidomycosis endemic areas of the southeastern United States become infected by *Coccidioides immitis.* While coccidioidomycosis has been reported in several different animal species (table 1), these reports do not indicate how prevalent the disease is in the general animal population. The purpose of this paper is to comment briefly on a series of animal surveys for *C. immitis* infections and to describe in detail the symptomatology and pathology of coccidioidomycosis in dogs, the animal most prone to develop symptoms.

## Observations

In the years 1954-1957, several thousand Arizona cattle were skin tested with undiluted coccidioidin*(fig. 1). It was found that in the most highly endemic areas of Arizona, almost 100 percent of the cattle became infected with *C. immitis* within their first two years of residence. Furthermore, Arizona slaughterhouse records indicate that approximately 250 cattle per month are found to have lesions of coccidioidomycosis.(11) It would appear that several million cattle in the coccidioidomycosis endemic areas of California, Arizona, New Mexico, Texas, Utah, and Nevada are infected at any given time.

A few horses, burros, and swine were also surveyed for coccidioidin reactivity. Thirteen of 22 horses raised near Phoenix, Arizona, gave positive reactions to undiluted coccidioidin. The average age of the horses was 3½ years. A burro from the vicinity of Mesa, Arizona, proved to be positive when tested. Seven of 65 swine with an average age of 8 months from Florence, Arizona, were positive.

Until a few years ago, only a scattered number of cases of canine coccidioidomycosis had been reported.(12-25) Most of these infections were disseminated, and the majority of them occurred in southern Arizona, particularly around Tucson. The disease is now known to be highly prevalent in dogs that live in endemic areas.

## Study on 100 Dogs

Between October 1954 and April 1956, I made post-mortem examinations of 100 dogs with disseminated coccidioidomycosis. All of these dogs came from a 50-mile radius of Phoenix, Arizona – a known endemic area for human and bovine coccidioidomycosis. The number of cases observed in man and cattle in this area during the study period fell within the normal range.

Most of the dogs were observed ante mortem, and pertinent clinical data were collected on all of them. In most cases, diagnosis was based on finding endosporulating spherules of *C. immitis* in lesion material on direct microscopic examination. In all the others, spherules containing endospores were demonstrated during examination of tissue sections. Also all cases were verified by isolation of *C. immitis* on Sabouraud's dextrose agar.

During this period other dogs with gross lesions typical of coccidioidomycosis were also examined. Smears from their lesions were found to be negative under direct microscopic examination, and time and facilities did not permit examination of tissue sections. However, in many cases *C. immitis* was isolated from lesion material by culture. During ante-mortem examinations of another group of dogs with disseminated lesions, spherules of *C. immitis* were demonstrated in smears of exudate from the lesions, and *C. immitis* was recovered by culture methods. No post-mortem examinations were made of these dogs. A number of other dogs with lesions suggestive of dissemi-

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*From the Communicable Disease Center, assigned Arizona State Department of Health, Phoenix.*

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*Lots 15087 and 59-62 kindly furnished by C. E. Smith, School of Public Health, University of California, Berkeley, California.

## TABLE 1

## RECORDED CASES OF COCCIDIOIDOMYCOSIS IN ANIMALS

| Animal Species | References | Animal Species | References |
|---|---|---|---|
| Cattle | 1-11 | Llama | 32 |
| Dog | 12-25 | Monkey | 33-34 |
| Horse | 26-27 | Gorilla | 33 |
| Burro | 28 | Chinchilla | 35 |
| Sheep | 29-31 | Wild Rodents | 36-37 |
| Swine | 32 | | |

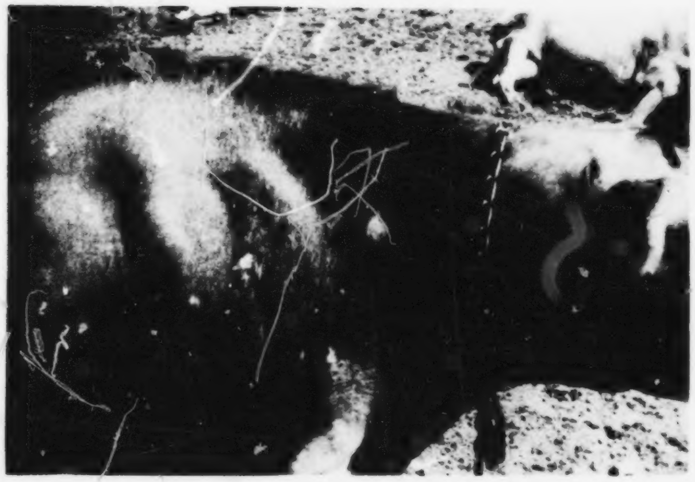

Figure 1. Positive coccidioidin skin test reaction of a cow with coccidioidomycosis.

nated coccidioidomycosis were offered for study, but lack of time prevented us from accepting a number of them for post-mortem examination. Obviously, the 100 confirmed disseminated cases we studied in detail represented only a portion of the cases occurring in the Phoenix area during the period. A number of nondisseminated cases were tentatively diagnosed by means of clinical observations. X-ray studies, skin tests, and precipitin tests.

The population of dogs above 6 months of age in the study area (Maricopa County) was estimated to be 73,000, with an average age of 4½ years.

### Age, Sex, and Breed

The ages and breeds of the 100 dogs under consideration are given in tables 2 and 3. The majority of disseminated coccidioidomycosis cases in this series occurred in dogs less than two years of age. When the popularity of various breeds in the area was considered in relation to the prevalence of coccidioidomycosis by breed in this study, Boxers and Doberman Pinschers appeared to be more susceptible to dissemination than other breeds. The mixed-breed dogs often resembled Boxers, Doberman Pinschers, or other dogs of the same genetic group. These two breeds are closely related genetically. After dissemination occurred, no statistically significant difference in the various symptoms and lesions was found in the different breeds.

Forty-one of the dogs studied were females and 59 were males, although the general proportion in the area is estimated at 36 females to 64 males. Of the 27 disseminated cases in dogs between 6 months and 1 year of age, 19 were in males and only 9 in females.

### Symptoms During Primary Illness

Twenty-two of the 100 dogs studied had no history suggestive of primary coccidioidomycosis. Respiratory illness typical of early nondisseminated coccidioidomycosis occurred in the other 78 from 2 weeks to 2½ years before symptoms of dissemination became evident. All of these had episodes of coughing ranging in duration from 1 week to 2 years, with a median time of 2 weeks. During the primary illness, 63 had fevers of 103° F. to 106° F., but usually about 104.5° F. Histories showed that antibiotics administered to 53 of these dogs failed to reduce the fever. Forty-two of the dogs had anorexia during the primary illness.

Careful observation, study, and use of serologic and skin tests now indicate that many dogs, like humans, acquire coccidioidomycosis, with or without apparent primary illness. The disease may proceed no further, and probably lifelong immunity to reinfection is thereby established.

### Symptoms of Dissemination

In man dissemination is said to occur in about 1 out of every 500 cases of primary infections. At present, the dissemination rate in dogs appears to be higher.

In this series of dogs the medium time at which dissemination became clinically apparent was four months after the primary illness started. In the majority of cases the primary respiratory illness, which lasted about 2 weeks, was followed by 3 to 5 months of apparent good health before dissemination became apparent. A few dogs showed symptoms of dissemination 2 or 3 weeks after primary symptoms started, with no intervening period of good health, whereas a few others appeared to be in good health for periods up to 2½ years between the primary and disseminated phases of their illness. Some dogs had intermittent periods of coughing and fever lasting a few days and others developed chronic coughs that persisted throughout the interval between the primary and disseminated illness.

Symptoms of dissemination varied considerably. Periods of coughing were common. The body temperature followed erratic courses, but often rose to about 104° F. daily. Some of the dogs had good appetites with only occasional periods of anorexia. A number that had large lung lesions experienced difficulty in swallowing. Twenty-two of the 45 dogs that lost weight during dissemination had chronic diarrhea, but only 5 of the others were so affected. Ascites was observed in 8 dogs. Lameness, which was often followed by atrophy of the unused leg muscles, was noted in 58 dogs (figs. 2, 3).

### Diagnostic Studies

X-ray studies were done on 14 dogs during the dissemination process. Chest radiographs revealed densities in the hilar area, constriction of the thoracic end of the trachea, dense areas around the large bronchi, and lesions of various sizes and numbers throughout the lungs (fig. 4). Bone radiographs revealed considerable new bone formation associated with some bone destruction (fig. 5).

Skin tests, using undiluted coccidioidin (lots

## TABLE 2

### AGE DISTRIBUTION OF 100 DOGS WITH CONFIRMED COCCIDIOIDOMYCOSIS

| Age | No. |
|---|---|
| 0 - 6 mo. | 9 |
| 6 mo. - 1 yr. | 27 |
| 1 yr. - 1½ yr. | 23 |
| 1½ yr. - 2 yr. | 17 |
| 2 yr. - 2½ yr. | 7 |
| 2½ yr. - 3 yr. | 8 |
| 3 yr. - 3½ yr. | 2 |
| 3½ yr. - 4 yr. | 3 |
| 4 yr. - 4½ yr. | 1 |
| 4½ yr. - | 3 |
| Total | 100 |

## TABLE 3

### DISTRIBUTION ACCORDING TO BREED OF 100 CANINE CASES OF COCCIDIOIDOMYCOSIS

| Breed | No. |
|---|---|
| Boxer | 39 |
| Mixed Breed | 16 |
| Doberman Pinscher | 11 |
| Boston Terrier | 6 |
| Fox and Rat Terriers | 6 |
| Collie | 4 |
| Cocker Spaniel | 4 |
| Beagle | 3 |
| Pointer | 3 |
| Shepherd | 3 |
| Dalmation | 2 |
| Great Dane | 1 |
| German Shepherd | 1 |
| Basset Hound | 1 |
| Total | 100 |

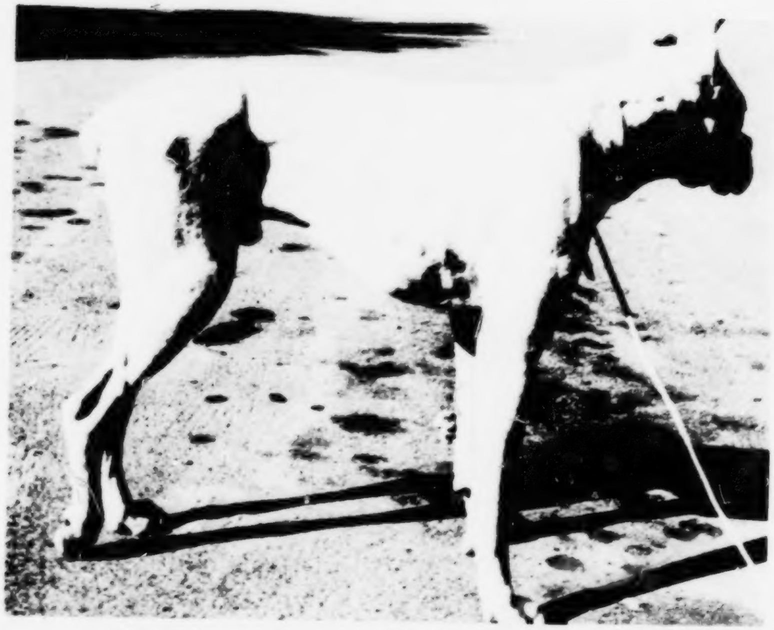

Figure 2. Dog with symptoms of early coccidioidomycosis dissemination. Note evidence of beginning lameness of the right rear leg; also positive reaction to skin test on the right side of the neck.

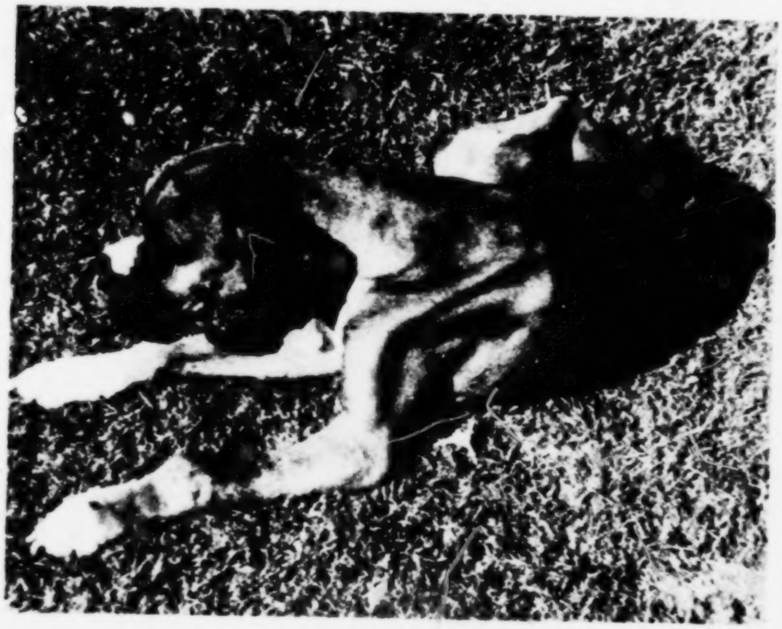

Figure 3. Dog with symptoms of extensive coccidioidomycosis dissemination. Note the cachexia, enlargement of the left carpus and stifle joint, and atrophy of the muscles of the left foreleg.

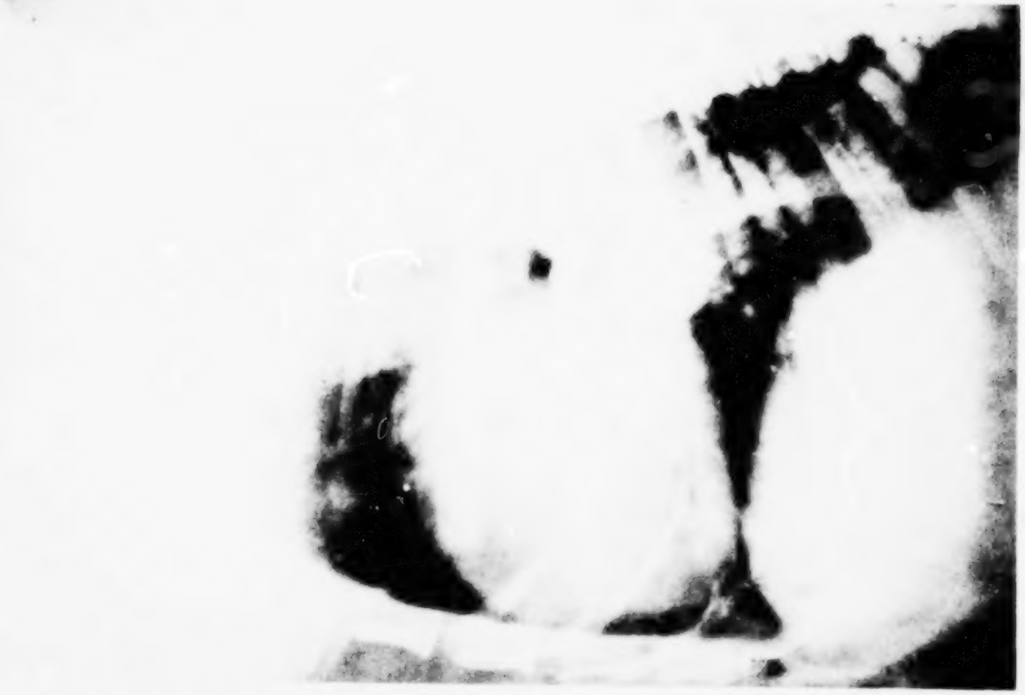

Figure 4. X-ray of pulmonary lesions in canine coccidioidomycosis. (*X-ray by R. Reed, D.V.M.*).

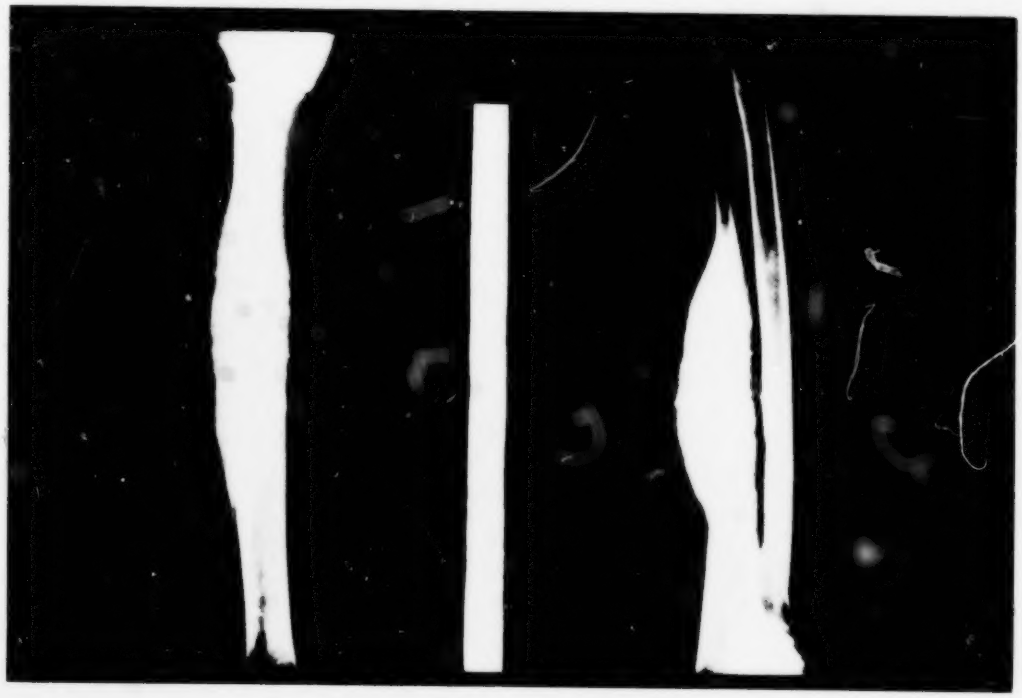

**Figure 5. X-rays of coccidioidal lesions of radius and ulna of a dog.** (*X-rays by B. Mariassy, D.V.M.*).

15087 and 59-62) furnished by C. E. Smith, were performed on 91 of the dogs (fig. 6). Positive tests were obtained on 70 dogs (76.9%), 21 (23.1%) of the dogs were negative. Some humans with disseminated infections also fail to give positive skin-test reactions.(38)

Precipitin tests were made on sera of 86 of the dogs. Fifty-one gave positive results and 35 were either negative or indefinite. Positive results were obtained more readily on sera drawn early in dissemination. The precipitin test furnished some slight indication of the extent of dissemination (table 4). At post-mortem 44 separate lesions classified as extensive were found in the 35 dogs that had given negative or indefinite precipitin-test results (1.26 lesions per dog); 86 separate, extensive lesions were found in the 51 dogs with positive reactions (1.7 lesions per dog).

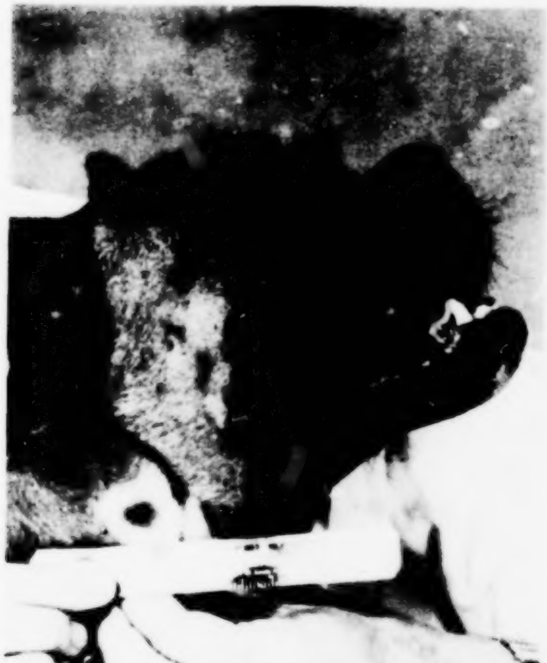

Figure 6. Positive coccidioidin skin test of a dog with coccidioidomycosis.

## Post-Mortem Observations

Most of the dogs were destroyed humanely with an overdose of an anesthetic when their condition became hopeless; the others died as a result of the disseminated disease. All were examined for grossly visible lesions.

The major lesions found in the soft tissues are listed in table 5. It was possible to correlate some of these and some of the untabulated lesions with signs and symptoms observed during the dissemination process. For example, pulmonary symptoms had been noted in 10 of the dogs later found to have slight lung lesions, in 22 of the 33 with well-marked lesions, and in 40 of the 44 with extensive lung lesions. At autopsy 19 of the dogs were found to have enteritis; all of these had had chronic diarrhea, and 17 of them had lost weight during dissemination. The 8 dogs with ascites all showed evidence of pericarditis; in 3 of these, lesions of the pericardium were slight and in 5, they were extensive.

In addition to the lesions listed in table 5, small separate subcutaneous lesions draining to the surface were observed in 3 dogs. It was also noticed that some leg-bone lesions of long duration produced sinuses draining through the skin. Two dogs had well-marked granulomas of the submaxillary lymph nodes; in one, this was associated with a well-marked lesion of the trachea. One dog had a granuloma in the peritoneal cavity, and another had a large granuloma in the periorbital fascia behind the eye. The fifth dog had grossly visible granulomas scattered throughout its entire body. This dog went blind two days before it was destroyed, and *C. immitis* was cultured from the vitreous humor.

The presence of bone lesions – particularly in the legs – is often obvious during the dissemination process. At autopsy 59 dogs had grossly visible lesions of the leg bones, usually near the epiphyseal junction (table 6). In some instances the lesions extended to all the bones in the joint area. During life 47 of the dogs had shown signs of lameness (table 7). Twenty-eight of the dogs with lesions in the leg bones had them in other bones as well (table 8).

The gross and microscopic pathology of coccidioidomycosis has been described adequately elsewhere.(12, 14-17, 24) It is very similar in man and dog, except in regard to bone lesions. In man the lesions tend to be more destructive in type, whereas in the dog they are more proliferative.

## TABLE 4

## CORRELATION OF PRECIPITIN REACTIVITY WITH EXTENT OF LESIONS IN 86 DOGS WITH DISSEMINATED COCCIDIOIDOMYCOSIS

| Organs and Tissues Affected | Number of Lesions in 35 Negative Precipitin Test Dogs | Number of Lesions in 51 Positive Precipitin Test Dogs |
|---|---|---|
| Lungs | 13 | 25 |
| Thoracic Lymph Nodes | 7 | 18 |
| Liver | 1 | 6 |
| Spleen | 2 | 4 |
| Kidney | 0 | 1 |
| Pericardium | 4 | 5 |
| Leg Bones | 8 | 24 |
| Other Bones | 7 | 3 |
| Other Tissues | 2 | 0 |
| Totals | 44 | 86 |

## TABLE 5

## MAJOR LESIONS FOUND IN SOFT TISSUES AT POST MORTEM OF DOGS WITH COCCIDIOIDOMYCOSIS

| Location | Extent | | | |
|---|---|---|---|---|
| | None | Slight | Well-marked | Extensive |
| Lungs | - | 23 | 33 | 44 |
| Thoracic Lymph Nodes | 8 | 45 | 18 | 29* |
| Liver | 10 | 67 | 15 | 8 |
| Spleen | 33 | 62 | 7 | 8 |
| Kidneys | 42 | 35 | 20 | 3 |
| Pericardium | 63 | 19 | 9 | 9 |
| Bladder | - | 2 | 1 | 7 |

* 25 of those had extensive lung lesions also.

## TABLE 6

### LOCATION OF OTHER OSEOUS LESIONS IN 59 DOGS WITH LEG BONE LESIONS CAUSED BY *COCCIDIOIDES IMMITIS* (SOME DOGS HAD MULTIPLE LEG BONE LESIONS)

| Fore Leg | | Rear Leg | |
|---|---|---|---|
| Bone or Joint Area | Number Affected | Bone or Joint Area | Number Affected |
| Scapula | 2 | Hip Joint | 4 |
| Shoulder Joint Area | 2 | Femur | 3 |
| Humerus | 3 | Patella | 1 |
| Elbow Joint Area | 1 | Stifle Joint Area | 5 |
| Radius | 3 | Tibia | 4 |
| Ulna | 4 | Fibula | 3 |
| Carpals | 22 | Tarsals | 7 |
| Metacarpals | 4 | Metatarsals | 7 |
| Phalanges | 2 | Phalanges | 4 |
| Totals | 43 | | 38 |

## TABLE 7

### RELATIONSHIP BETWEEN LAMENESS AND OCCURRENCE OF LEG BONE LESIONS IN 100 DOGS WITH DISSEMINATED COCCIDIOIDOMYCOSIS

| | Number of Dogs | Extent of Bone Lesions | | | Number of Dogs With Visible Bone Lesions |
|---|---|---|---|---|---|
| | | Slight | Well-Marked | Extensive | |
| Lameness | 58 | 8 | 6 | 33 | 47 |
| No Lameness | 42 | 2 | 0 | 10 | 12 |
| Totals | 100 | 10 | 6 | 43 | 59 |

## TABLE 8

## COCCIDIOIDOMYCOTIC BONE LESIONS OTHER THAN IN THE LEG FOUND AT POST MORTEM IN 28 DOGS WITH LEG BONE INVOLVEMENT

| Location | Extent | | |
|---|---|---|---|
| | Slight | Well-marked | Extensive |
| Ribs | 3 | 5 | 1 |
| Mandible | 1 | 2 | |
| Pelvis | | | 3 |
| Lumbar Vertebrae | | 2 | |
| Thoracic Vertebrae | | 1 | |
| Cervical Vertebrae | | 1 | |
| Skull | | 1 | |
| Sacrum | | 1 | |
| Ribs and Pubis | | 1 | 1 |
| Ribs and Ilium | | 1 | 1 |
| Ribs and Lumbar Vertebrae | | | 1 |
| Pelvis and Mandible | | | 1 |
| Ribs, Ilium, and Sacrum | | | 1 |

Although only a small amount of tissue sectioning was done in the present study, microscopic lesions were no doubt present in many areas where there were no gross lesions.

**Infectivity Studies**

In general, the infected mammal is not considered to be a direct threat to other mammals. However, studies now in progress on dogs with disseminated and nondisseminated cases of coccidioidomycosis indicate that some of these animals release large numbers of spherules in their saliva, urine, feces, and wound exudates, and that infective arthrospores develop within a few days on the contaminated soil. Although this may not provide a substantial reservoir of infection insofar as public health is concerned, possible risk to the dog owner and his family may have to be considered.

**Summary**

The clinical signs and pathology of canine coccidioidomycosis in 100 confirmed cases are described. A tabulation of all recorded wild and domestic animal infections caused by *Coccidioides immitis* is presented along with personal observations of coccidioidomycosis in cattle, horses, burros, and swine.

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## *Section IV*

# Epidemiologic And
# Ecologic Features
# Of Coccidioidomycosis

# Coccidioidin, Histoplasmin, and Tuberculin Sensitivity of Students in Selected High Schools and Colleges in Arizona

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Patrick H. Lehan, M.D., Harry Rubin, M.D., J. C. Greene, D.M.D.,
E. Casper, R.N., and Paul J. Coleman, M.D.

In cooperation with the Arizona State Department of Health, County Health Units, and local school personnel, a team from the Communicable Disease Center of the Public Health Service conducted skin test surveys of students in 2 high schools and 4 colleges in Arizona in September 1956.

The purpose of the survey was to determine the levels of sensitivity to coccidioidin of persons from different parts of the State. The two high schools were included to offset the large number of out-of-state residents expected among the college students.

At a later date we hope to conduct spot surveys in counties where insufficient numbers of students were tested. Therefore this report should be considered as preliminary.

## Material and Methods

Approximately 3400 students at 4 Arizona colleges and 2 high schools were tested at the beginning of the school year in September. Most of them were between 13 and 24 years of age. At the colleges, the intradermal tests were given to all incoming freshmen as part of the entrance physical examination. At the high schools, the tests were given to all students whose parents consented. They constituted an unusually high percentage of the student body. Prior to the testing, each student filled out a questionnaire which included identifying data and a detailed residence history listing in chronological order all places in which the student had resided for 6 months or more.

Intradermal tests using coccidioidin, tuberculin, and histoplasmin as antigens were administered on the volar surfaces of the forearms and read at 48 hours. Tests were read by measuring the transverse diameters of both erythema and induration in millimeters. A test was considered positive if the induration measured 5 mm. or more in diameter. The concentrations of antigens used were intermediate strength PPD (.0001 mg.), 2:1000 dilution of histoplasmin Lot HKC 5, and 1:100 dilution of coccidioidin Lot 64 D5. The latter was obtained from Dr. C. E. Smith of the University of California at Berkeley.

## Results

The gross sensitivity to the three antigens is shown in table 1. That only 41 percent of the persons tested were positive to coccidioidin was probably due to the fact that only a third of them could be considered lifetime residents of Arizona. The tuberculin rate among the whites was 12 percent, which is considerably higher than the national average,(1) (8.8 percent). The rate among the nonwhites, who were predominantly Negroes and Indians, was 2½ times that of the whites in the survey (29 percent). The significance of the 30-percent histoplasmin will be considered in the discussion.

In an effort to determine the prevalence of positive reactors to coccidioidin, only 80-percent-lifetime residents were considered. An 80-percent-lifetime resident was defined as any person spending this portion of his life in a single county and who had not resided in any area outside the State endemic for either coccidioidomycosis or histoplasmosis. Table 2 shows the prevalence of reactors by counties for the 955 persons who met this criterion. The coccidioidin sensitivity rates were more than 70 percent among the residents of Pima, Pinal, and Maricopa counties and 60 percent

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*From the Communicable Disease Center, assigned Arizona State Department of Health, Phoenix; CDC Kansas City Field Station, Kansas City, Kansas; Los Angeles County Health Department, Los Angeles, and the CDC Phoenix Field Station, Phoenix*

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## TABLE 1

## PREVALENCE OF SENSITIVITY TO COCCIDIOIDIN, HISTOPLASMIN, AND TUBERCULIN BY RACE IN VARIOUS ARIZONA SCHOOLS

| Race | Number Tested | Number Positive | | | Percent Positive | | |
|---|---|---|---|---|---|---|---|
| | | Coccidi-oidin | Histo-plasmin | Tuber-culin | Coccidi-oidin | Histo-plasmin | Tuber-culin |
| White | 3245 | 1324 | 1010 | 385 | 40.8 | 31.2 | 11.7 |
| Non-White | 153 | 69 | 52 | 45 | 45.1 | 34.0 | 29.4 |
| Total | 3398 | 1393 | 1062 | 430 | 41.0 | 31.2 | 12.7 |

## TABLE 2

## PREVALENCE OF COCCIDIOIDIN SENSITIVITY AMONG 80-PERCENT-LIFETIME RESIDENTS OF ARIZONA BY COUNTY

| County | Number Tested | Number Positive | Percent Positive |
|---|---|---|---|
| Apache | 23 | 3 | 13 |
| Cochise | 27 | 10 | 37 |
| Coconino | 21 | 1 | 5 |
| Gila | 31 | 15 | 48 |
| Graham | 235 | 141 | 60 |
| Greenlee | 10 | 4 | 40 |
| Maricopa | 350 | 247 | 71 |
| Mohave | 7 | 4 | 57 |
| Navajo | 31 | 5 | 16 |
| Pima | 145 | 105 | 72 |
| Pinal | 29 | 22 | 76 |
| Santa Cruz | 5 | 2 | 40 |
| Yavapai | 14 | 2 | 14 |
| Yuma | 27 | 7 | 26 |
| TOTAL | 955 | 568 | 59 |

in Graham County. All of these counties are located in the southern part of the State. In contrast, the rates were 5 to 16 percent in the northern and northeastern counties. The general geographical distribution agreed with previous studies.(2)

The prevalence of coccidioidin reactors by counties showed close agreement with the natural geographical division within the State. If, as is shown in figure 1. Arizona were divided into two parts according to the natural division between desert and mountain country, the counties with high prevalence rates would fall within the Lower Sonoran Life Zone. This zone is primarily desert country characterized by alkaline soil, relative freedom from severe frosts, and very hot, dry seasons of several months duration followed by some rains. The annual rainfall is from 3 to 20 inches. These conditions presumably are ideal for the propagation of *Coccidioides immitis* in nature: July mean temperature about 90° F., January mean temperature 51° F., and annual rainfall from 5 to 15 inches. In contrast, the northern half of the State is for the most part mountainous with considerably more rainfall and lower room temperatures during the year.

The 80-percent-lifetime residents are grouped according to positive and negative coccidioidin and histoplasmin reactions in table 3 as compared to similar figures compiled by Edwards and Palmer.(2) Twenty-five percent of those studied had positive reactions to both antigens. Most of the 34 persons reacting to histoplasmin alone were found in the southern counties and comprised less than 5 percent of the persons tested there.

The distribution of persons in the four reactor groups is markedly different from that of Edwards and Palmer.(2) Thirty-seven percent failed to react to either antigen compared to 85 percent in the earlier study; and 25 percent reacted to both antigens compared to the 4 percent obtained previously. A possible source of the difference in results may have been the fact that our study was conducted in a highly endemic area. Interestingly enough, the distribution of 1,030 short-term residents who had not resided in other endemic areas was similar to that of the 80-percent-lifetime residents in the same study rather than to that of Edwards and Palmer.

The frequency distribution of histoplasmin reactions by size of induration for 3 groups of coccidioidin reactors among the 955 80-percent-lifetime reactors agreed with Edwards and Palmer's findings rather closely if the reactors were divided into 3 groups by their coccidioidin reaction: the essentially negative with reactions of 0-4 mm. induration; the minimal positives with reactions of size 5-10 mm.; and the positives with reactions 10 mm. or greater. The mean reaction size for positive histoplasmin reactions $\geq 3$ mm. was consistently higher than in Edwards and Palmer's group. Surprisingly enough, a similar distribution was observed among the 1,030 short-term residents.

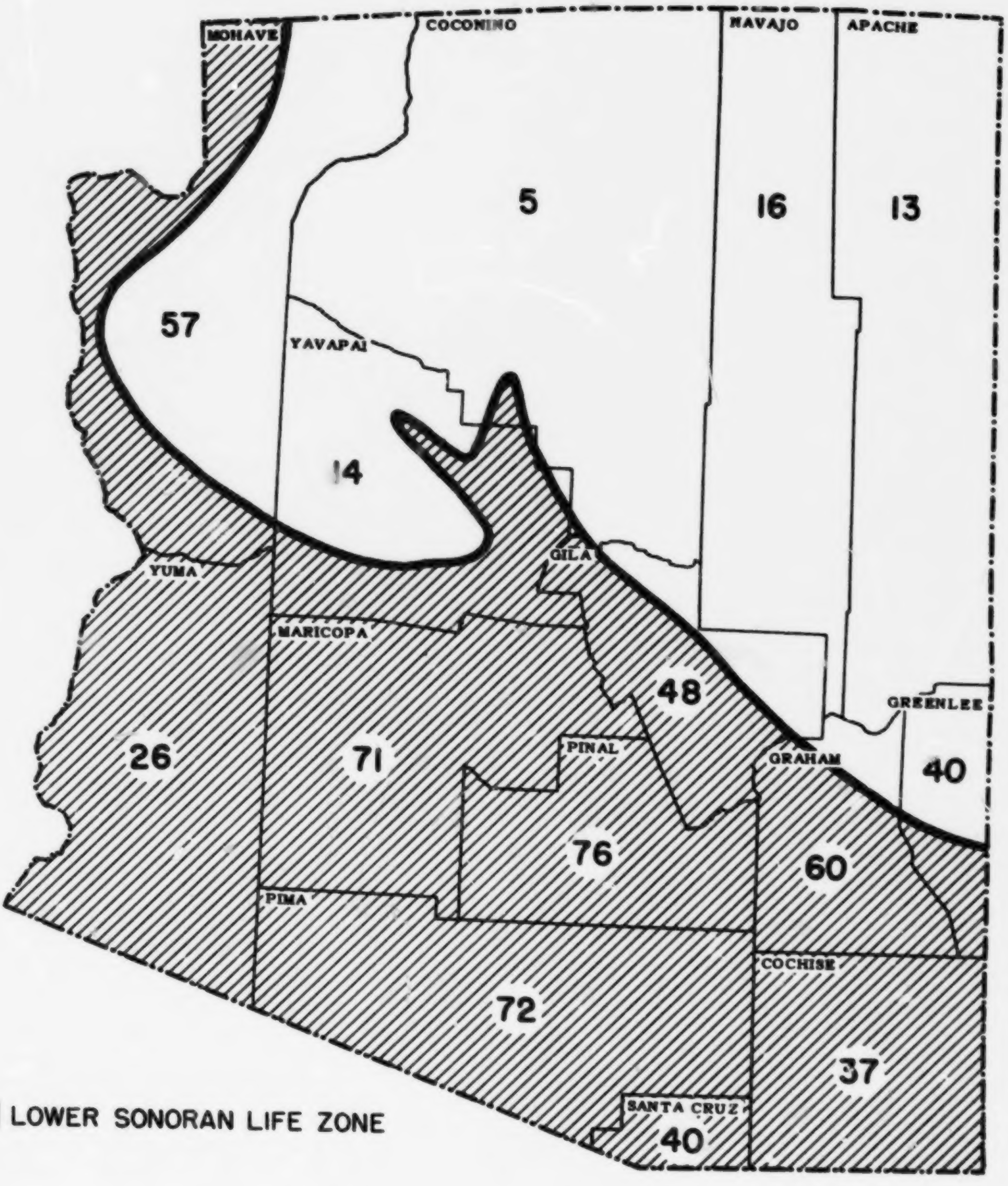
If the reverse classification according to histoplasmin reaction was made among the 955 80-percent-lifetime residents, the reactions to coccidioidin increase in size with the increase of the size of the histoplasmin reaction, the means of reactions $\geq 3$ mm. being 10.1 mm., 11.9 mm., and 14.8 mm., respectively, for reactors with 0-4 mm. reactions, 5-10 mm., and $\geq 11$ mm. to histoplasmin. This was not found among the 1,030 other Arizonans. Determination of annual conversion rates of coccidioidin reactors was based on the length of time a person had lived in a single county without having been in other endemic areas. Residents of Pima, Pinal, and Maricopa counties were grouped together because of the similarity in environment and prevalence. This grouping was used for the residents of Apache, Navajo, Coconino, and Yavapai counties. Graham county residents were considered separately. Only the first 12 years of residence was employed because a tendency for the prevalence to level off after 12 years of exposure was observed. The annual conversion rates for each area were computed by a method outlined by Manos(3) and were, respectively, 10 percent per year for the 3 southern counties, 7 percent per year for Graham county, and 1 percent per year for the northern counties.

## Discussion

As noted above, the prevalence and annual conversion rates of coccidioidin reactors were high in the southern half of the State and low elsewhere. These facts correlated very well with the ecological studies in progress which indicate that the ideal climate for *C. immitis* is found in the area of high prevalence and coincides with the Lower Sonoran Life Zone.(4) Furthermore, repeated testing of negative reactors among persons and animals has shown that conversion is more rapid in a highly endemic area than is indicated from a study of prevalence. Periodic surveys(5,6) in selected areas of Maricopa, Pima, and Pinal coun-

## FIGURE 1

## PREVALENCE OF COCCIDIOIDIN SENSITIVITY AMONG 80% - LIFETIME RESIDENTS OF ARIZONA BY COUNTY


MOHAVE
COCONINO
NAVAJO
APACHE
5
16
13
57
YAVAPAI
14
GILA
YUMA
MARICOPA
48
GREENLEE
PINAL
GRAHAM
26
71
40
76
60
PIMA
COCHISE
72
37
SANTA CRUZ
40
▨ LOWER SONORAN LIFE ZONE

JUNE 1957

## TABLE 3

### COMPARISON OF REACTIONS TO COCCIDIOIDIN (C) AND HISTOPLASMIN (H) AMONG NAVY RECRUITS FROM SOUTHWEST STATES AND ARIZONA STUDENTS

| Reactor Group | Navy Recruits From Southwest. Lifetime Residents of One County* | | Arizona Students | | | |
|---|---|---|---|---|---|---|
| | | | 80-Percent-Lifetime Residents | | Other** | |
| | Number | Percent | Number | Percent | Number | Percent |
| C+, H+*** | 256 | 4.3 | 242 | 25.3 | 209 | 20.3 |
| C+, H– | 405 | 6.8 | 326 | 34.1 | 257 | 25.0 |
| C–, H+ | 232 | 3.9 | 34 | 3.6 | 71 | 6.9 |
| C–, H– | 5060 | 85.0 | 353 | 37.0 | 493 | 47.8 |
| Total Tested | 5953 | 100.0 | 955 | 100.0 | 1030 | 100.0 |

* Figures obtained from Edwards and Palmer: Prevalence of Sensitivity to Coccidioidin, etc. PHR, 71: July 1956.

**Short-term residents of Arizona who have not resided in other endemic areas.

*** Positive reaction "+" is defined as one with induration 5 mm. or more in transverse diameter. Negative reaction "–" is defined as one with induration less than 5 mm. in diameter.

ties have indicated that most cattle will have converted to a positive reaction within 2 years and most humans within 5 years. Persons living close to the soil under very primitive conditions and cattle in the same area convert at approximately the same rate. Those living under average conditions with average homes that are reasonably dustproof and with grassy yards have conversion rates of about half those of cattle in the same general area. The rate of persons living in residential areas which are separated from the surrounding countryside by a buffer zone of trees, grass, and paved streets is half that of persons who live in average homes in average areas. The tendency for people to travel from place to place is also a hindrance in use of the skin test as an indicator for endemicity. Therefore, caution should be exercised in interpreting the prevalence of positive reactors, since the large majority of persons tested came from urban surroundings. The apparent leveling off of sensitivity after long exposure should also be kept in mind.

Cross-reactions between histoplasmin and coccidioidin have long been a problem to workers in this field.(2,7) It is possible that the high percentage of histoplasmin reactors noted – 29 percent of the 80-percent-lifetime residents – was due to this phenomenon, although some could be due to infections acquired during short trips into histoplasmosis-endemic areas that were not recorded in the residence histories. With few exceptions, the histoplasmin reactors, both those who reacted to coccidioidin and those who did not, were found in the area highly endemic for coccidioidomycosis. This would indicate either that endemic foci of histoplasmosis exist in southern Arizona or that the incidence of positive histoplasmin reactions increases with the prevalence of positive coccidioidin reactions.

## Summary

Thirty-four hundred students in 6 Arizona schools were skin tested with coccidioidin, histoplasmin, and tuberculin. The over-all tuberculin rate of 12.7 percent is higher than the national average for this age group. Particular attention is directed to coccidioidin reactors among 955 of these students who had spent 80 percent of their lifetime in Arizona and had never resided in out-of-State areas known to be endemic for coccidioidomycosis or histoplasmosis. Counties in south-central Arizona showed the greatest percentage of coccidioidin reactors with 71 percent positive in contrast to the counties in the north and northeast portion of the State with 5 to 16 percent positive. The significance of the reactions to histoplasmin is discussed. As indicated earlier, future tests are planned for the inadequately surveyed areas.

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# Skin-Test Survey at Williams Air Force Base, Arizona

Paul G. Hugenholtz, M.D.

From February 1953 to March 1954, a skin test survey for coccidioidomycosis was conducted at Williams Air Force Base, Chandler, Ariz., as part of a larger survey at Air Force bases situated within the endemic coccidioidomycosis areas (table 1). In an attempt to establish the present degree of coccidioidomycosis prevalence at Williams Air Force Base, a similar survey was conducted between November 7, 1955 and November 7, 1956.

The problem of the exact geographic demarcation of coccidioidomycosis has remained of prime importance. Since the southwestern part of the United States possesses unlimited desert ranges for training of ground troops and advantageous climatic conditions for year-round flying, it has remained of considerable interest to the military. Several of the bases originally established during World War II have been kept on a semi-permanent or permanent basis. Williams Air Force Base near Phoenix is one of these.

Exact incidence rates for coccidioidal infections among Air Force personnel were not available during World War II except for a few bases in Arizona and in the San Joaquin Valley of California. Smith *et al.*(1) concluded that 25 to 50 per hundred susceptibles were infected at these installations. When one realizes that 40 percent of all cases are symptomatic infections and that, if hospitalized, the average time lost per case ranges between 30 and 60 days,(1,2) the importance of the problem during World War II may be appreciated.

From January 1952 until November 1956 an average of 60 persons with clinical cases of coccidioidomycosis were hospitalized at Williams Air Force Base per year. The total average strength during this period varied between 2,000 and 3,000 military personnel.

From 1951-1956, the average time loss per year because of coccidioidomycosis was 2,113 man-days; for accidents of various kinds, 1,238 man-days; and for tonsillitis, 311 man-days (fig. 1).

No further pertinent work in this field has been done since results of the skin test survey were published in December 1954 by the United States Air Force, School of Aviation Medicine.(3) The present data were compiled in order to get an impression of the relative prevalence of the disease at Williams Air Force Base and of the seasonal incidence of skin test conversion. The study period was therefore divided into 4 three-month intervals. Retesting was done only on individuals who were negative at the previous testing.

## Methods and Materials

A group of permanent personnel, chiefly in maintenance and supply, who arrived on this Base on or about November 7, 1955, during "change-over" in mission received a series of intradermal tests with coccidioidin and histoplasmin (table 2). The first testing took place on February 7, 1956, approximately 11 weeks after the men's arrival at the Base. The second test was on May 7, the third on August 14, and the fourth on November 7. The periods were chosen in order to "schedule" suspected seasonal peaks just prior to the next testing date.

Commercially prepared coccidioidin was used in a 1:100 dilution. Histoplasmin, also prepared commercially, was used in a 1:1000 dilution. All antigens were diluted just prior to administration. The tuberculin syringes and 26-gauge needles were cleaned in a potassium dichromate solution to prevent cross-reactions. The skin tests were performed by injecting 0.1 cc. of the appropriate dilution of each antigen intracutaneously on the lower volar aspect of both forearms.

A reading was done 24 and 48 hours after injection, mainly by one observer, and recorded as the actual measurement of cross-section of the area of induration. The size of reaction was interpreted in terms of generally accepted symbols such as: ± for erythema alone or with induration less than 5 mm., + for induration with diameter of 5 to 9 mm., ++ for induration with a diameter of 10 to 19 mm.,

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*From the U. S. Air Force Hospital, Williams Air Base, Arizona, now Boston City Hospital, Boston, Massachusetts.*

## TABLE 1

## COCCIDIOIDIN SENSITIVITY SURVEY, WILLIAMS AIR FORCE BASE

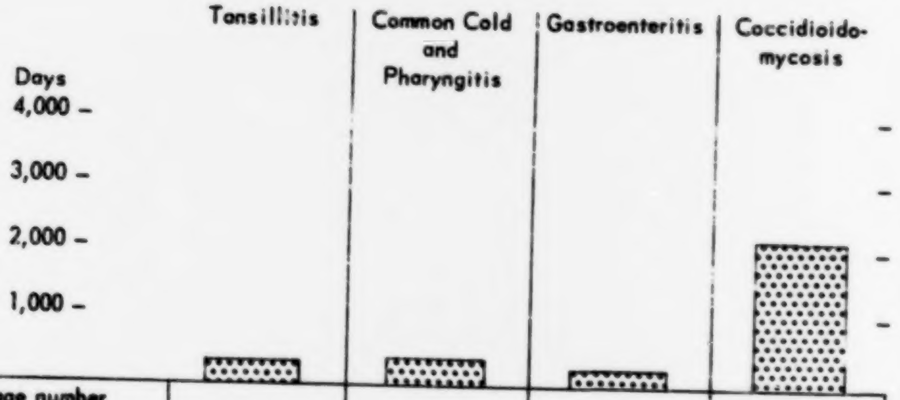
## 1953-1954

| Month, Year | Interval between Skin Tests | Number Tested | Number Positive | Percent Positive |
|---|---|---|---|---|
| February 1953 | | 1,138 | 178 | 15.6 |
| July 1953 | 28 weeks | 385 | 58 | 15.1 |
| March 1954 | 27 weeks | 106 | 22 | 20.8 |
| 1954 Over-all Conversion Rate: 310/1000 Susceptibles per Year | | | | |

Figure 1

MORBIDITY DUE TO COCCIDIOIDOMYCOSIS
AND OTHER DISEASES
(Williams Air Force Base)

AVERAGE DAYS LOST PER YEAR
1952 – 1956



| | Tonsillitis | Common Cold and Pharyngitis | Gastroenteritis | Coccidioidomycosis |
|---|---|---|---|---|
| Average number days lost per year | 311 | 324 | 208 | 2,113 |
| Average number cases per year | 54 | 61 | 44 | 61 |
| Average number days lost per case | 5.8 | 5.3 | 4.7 | 34.6 |

## TABLE 2

## RESULTS OF COCCIDIOIDIN AND HISTOPLASMIN TESTS PERFORMED IN 1956

| Date (1956) | Interval in Weeks | Number Tested | Coccidioidin Positive | | Histoplasmin Positive | | Histoplasmin Converters | |
|---|---|---|---|---|---|---|---|---|
| | | | Number | Percent | Number | Percent | Coccidioidin Positive Group | Coccidioidin Negative Group |
| February 7* | | 319* | 38** | 11.9 | 108† | 33.8 | | |
| May 7 | 13 | 205 | 11 | 5.3 | 89 | 34.1 | 8 | 0 |
| August 14 | 14 | 192 | 8 | 4.1 | 65 | 33.7 | 2 | 1 |
| November 7 | 13 | 159 | 15 | 9.4 | 55 | 34.5 | 6 | 2 |
| Total Available for Entire Coccidioidomycosis Survey | | 188 | 44 | 23.4 | | | | |
| 1956 Over-all Conversion Rate: 234/1000 Susceptibles per Year | | | | | | | | |

*Tested 11 weeks after arrival at Williams Air Force Base.

** 196 previously had been in the endemic area; 123 never before in an endemic area.

***28 of the 38 were previously in the endemic area.

†78 were born or raised in an area endemic for histoplasmosis.

+++ for induration of the diameter of 20 mm. or more and ++++ for vesiculation and necrosis.

All subjects were males between 17 and 45 years of age, with the majority between 20 and 30 years of age. Individual records kept for each subject included identifying data such as: name, serial number, State of birth, and a note as to whether or not the individual had resided for one or more months within an endemic area for coccidioidomycosis or had traveled through one.

At the time of the last retesting, chest roentgenograms and serologic tests for presence of complement fixation and precipitin antibodies were made on all "converters."

### Results

In the initial coccidioidin and histoplasmin tests performed February 7, 1956, 38 of the 319 men tested were coccidioidin positive. However, 28 of these reactors had previously been in an endemic area and were not considered further in this series. The 10 coccidioidin positives among the remaining 291 (2.9 percent) were thought to represent true infections, since they had only been in the endemic area since November 7, 1955. In the total group, 108 (33.8 percent) histoplasmin positives were found, and of these, 30 were neither born nor raised in the endemic area for histoplasmosis. They may have been infected, however, during military or civilian travel through this area.

In May, after an interval of 13 weeks, 205 permanent party personnel were retested. Eleven (5.3 percent) of them had become coccidioidin positive. Of the total group, 70 (34.1 percent) were found to be positive for histoplasmin. Nearly all of these had had a positive histoplasmin skin test previously. Eight of the histoplasmin positives who had been negative 13 weeks previously belonged to the coccidioidin negative group, and 6 of these came from an area endemic for histoplasmosis. There were no histoplasmin converters in the coccidioidin positive group.

In August, after a second interval of 14 weeks, 192 of the original group were subjected to second retests. Eight (4.1 percent) of this group now were coccidioidin positive, and 65 (33.7 percent) of them were histoplasmin positive. There were 3 histoplasmin converters, 2 of whom belonged to the coccidioidin negative group. Both of these came from the histoplasmosis endemic area. The third histoplasmin converter, who was found in the coccidioidin positive group, also came from the endemic area.

Thirteen weeks later, in November, retests were done for the third time on 159 men. Fifteen (9.4 percent) of these had now become coccidioidin positive. There were two histoplasmin converters in the coccidioidin positive group. Neither came from the histoplasmosis endemic area.

A group of 188 men was available for repeated retesting from November 7, 1955, until November 7, 1956. Among these, 44 (23.4 percent) became coccidioidin positive during the year.

Although the groups retested at the various intervals did not always comprise the same individuals as the original 319, a persistent, similar percentage of histoplasmin positives was found. This averaged 34 percent. Ten persons became histoplasmin positive while not converting to coccidioidin. Six of these came from the endemic area for histoplasmosis. Three persons converted to histoplasmin positive while their coccidioidin skin tests became positive at the same time. Five of the group tested on November 7, 1956, had "lost" the sensitivity to histoplasmin they evinced on February 7, 1956.

Of the 11 coccidioidin converters in the May test, 5 had been histoplasmin negative previously and 6 had been positive. Of the 8 coccidioidin positives in the August test, 4 had been histoplasmin negative and 4 had been positive. Finally, 11 of the 15 coccidioidin converters in November previously belonged to the histoplasmin negative and 4 to the histoplasmin positive group.

Of the 44 individuals in this survey who converted from coccidioidin negative to positive during the year of study, only one had a clinically recognized case and was hospitalized. The remaining 43, using Smith's *et al.*(1) San Joaquin Valley report (1943-1945) as a basis, should be classified as "inapparent" cases. All converters were questioned as to possible symptomatology. Only five admitted having had any symptoms and these were men who had had a "cold" associated with chest pain. However, their descriptions were so vague and the course of the disease was of such short duration that they were classified as "inapparent" also. This does not correspond with Smith's observation that symptomatic, clinically recognized cases constitute approximately one-fourth of all primary coccidioidal infections. It does seem to coincide, however, with the unusual low incidence of clinical cases observed during 1956 at Williams Air Force Base Hospital. The usual incidence rate is 60 cases per year, and to date only 18 cases have been observed. Speculation about this observation is wide open. The percentage of skin test conversions does not seem to be much lower than in the 1953-1954 series, thereby suggesting a possible variation in virulence of *C. immitis* rather than in incidence of coccidioidomycosis.

As far as the seasonal incidence is concerned the 9.4 percent in the group tested during the fourth trimester is nearly double that of the previous periods. This correlates well with the clinical impression that there is a peak of the disease in the fall. Forty-six (35.6 percent) of the 129 clinically recognized cases during 1953 were seen during the September-November trimester. In past years a similar trend has been observed here and in the San Joaquin Valley of California.

During the second trimester of the survey, Dr. K. T. Maddy, veterinarian with the U. S. Public Health Service,(4) tested 105 head of one-year-old Brahman and Santa Gertrudis cattle in a feed lot at the western edge of Williams Air Force Base. He found 104 were coccidioidin negative. Three months later when he retested 80 of the previously negative cattle, he observed 13 "converters" (16.2 percent). The conversion rate for cattle is estimated to be twice that of man. If this percentage in cattle remains consistent for more than one year, it would compare with that observed in our human series.

At the time the last retesting was done on Air Force personnel, roentgenographic examinations and serologic tests for antibodies were performed on nearly all of the coccidioidin converters.

Thirty-five of the 44 coccidioidin converters in the study were available for roentgenographic examination of the chest at the termination of the experiment. Twelve demonstrated residual solitary nodules measuring between 0.5 and 1 cm. in diameter. The chest X-rays of nearly all of these had previously shown normal findings with no cavities. Of the 23 converters available for serologic testing, 15 were converters as of the November 7, 1956, test, while the remainder had converted earlier and had nodular residuals. Only three had diagnostically elevated complement fixation antibodies, and each of these had nodular residuals.

In this small series, residual lesions were demonstrable in 34.2 percent of those converters available for roentgenographic examination. All had typical nodular residuals. Only one of these men had a clinically recognized case of coccidioidomycosis. Although the smallness of this series does not allow a statistical analysis, one can assume that between one-fourth and one-third of the asymptomatic skin test conversions are accompanied by demonstrable residual pulmonary lesions, mainly in the form of nodules which are detectable only on close examination.

## Summary

A group of newly arrived, permanent personnel with negative reactions to intradermal coccidioidin was followed at three-month intervals with coccidioidin and histoplasmin skin tests. Forty-four individuals out of 188 became positive within a one-year period. Approximately 30 percent of them had demonstrable residual pulmonary lesions and 25 percent of these individuals had demonstrable antibodies in their serum. Only one of these men had a clinically recognized case of coccidioidomycosis and was hospitalized.

This series demonstrates that during 1956 the infection rate was 23.4 percent. A previously suspected seasonal peak in infection rate in the fall is supported by a nearly doubled conversion rate during the period August 14 to November 7, 1956.

## References

(1) Smith, C. E., Beard, R. R., Rosenberger, H. G., and Whiting, E. G.: Effect of season and dust control on coccidioidomycosis. J. A. M. A. 132:833-838, 1946.

(2) Smith, C. E.: Coccidioidomycosis. Pediat. Clin. North America 2:109-125, 1955.

(3) U. S. A. F. School of Aviation Medicine: Studies on coccidioidomycosis at air force bases in southwestern United States. U. S. A. F. School of Aviation Medicine, Randolph Field, 1954, 25 pp.

(4) Maddy, K. T.: Personal communication.

# Comparative Study of Time Loss in Coccidioidomycosis and Other Respiratory Diseases

John T. Scogins, M.D.

The purpose of this study is to compare the number of man-days lost by personnel at the Luke Air Force Base, Glendale, Ariz., because of hospitalization due to coccidioidomycosis with those lost because of other highly prevalent diseases. The data used were abstracted from records of the 3600th USAF Hospital and include all cases of coccidioidomycosis and of the disease groups selected for comparison that occurred between May 1951, when the hospital was reactivated, and December 31, 1956. The present report is merely a brief summary of our findings.

Three disease groups comprised of the upper respiratory infections, the pneumonias, and infectious mononucleosis were selected for comparison with coccidioidomycosis. The upper respiratory group included tonsillitis, pharyngitis, common colds, and undifferentiated upper respiratory infections. All acute pneumonias were included in the second group. Many of these were unclassified as to type, and there is reason to believe that a number of them were actually undiagnosed coccidioidomycosis. Infectious mononucleosis was chosen for the third group under comparison because it represents one of the major causes of time loss at this Air Base.

An average of 70 days per year were lost because of coccidioidomycosis between May 1951 and January 1954, with 5.6 cases each year requiring an average of 12 days hospitalization (fig. 1). The actual extent, however, was probably much greater, since 226 of the 452 days lost annually because of the pneumonias were charged to "unclassified" types of the disease, and many of the latter were undoubtedly undiagnosed coccidioidomycosis.

Between January 1954 and December 31, 1956, the average annual time lost because of coccidioidomycosis increased to 1,063 days, with an average of 32 patients hospitalized each year for 33.2 days (fig. 2). The number of man-days lost per case corresponded with the experience at Williams Air Force Base, Chandler, Ariz., where each case required 34.6 days hospitalization. During the same period in our study, the total time loss due to unclassified pneumonias dropped to an average of 62 days per year as compared to 226 days per year during the preceding period. This variation can be explained easily. By 1954 the population at the Air Base had become more stable and had been exposed to *Coccidioides immitis* for a longer period of time. Also, our diagnostic acuity and knowledge of coccidioidomycosis had progressed so that fewer cases of this disease were misdiagnosed as pneumonia. At this Air Base, coccidioidomycosis was exceeded only by trauma as a cause of days lost during this second study period.

Since 1952 the number of days lost per 1,000 personnel has increased annually as follows: 11 in 1952, 24 in 1953, 227 in 1954, 238 in 1955, and 273 in 1956. The figure for 1956 may present the most accurate picture of what to expect in the future since awareness of the disease and means of detecting it have become more highly developed.

It is well known that members of the dark-skinned races are more susceptible than light-skinned people to coccidioidomycosis. In our study, 24.1 percent of the patients hospitalized for coccidioidomycosis were Negroes. Only 9.1 percent of the patients hospitalized for the other diseases under consideration were Negroes.

There was a clearly defined seasonal variation in the incidence of coccidioidomycosis, with the peak following the rainy seasons (fig. 3). Seasonal variation was less marked in regard to the upper respiratory infections and the pneumonias.

## Summary

Coccidioidomycosis is now the major medical problem among personnel at the Luke Air Force Base, Glendale, Ariz. Its increasing incidence, seasonal variations, and predilection for dark-skinned races are compared with those for other selected groups of disease.

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*From the 3600th U. S. Air Force Hospital, Luke Air Force Base, Glendale, Arizona, now at Jefferson Davis Hospital, Houston, Texas.*

FIGURE 1

COMPARATIVE INCIDENCE OF COCCIDIOIDOMYCOSIS AND OF SELECTED DISEASES
MAY 1951 - JANUARY 1954

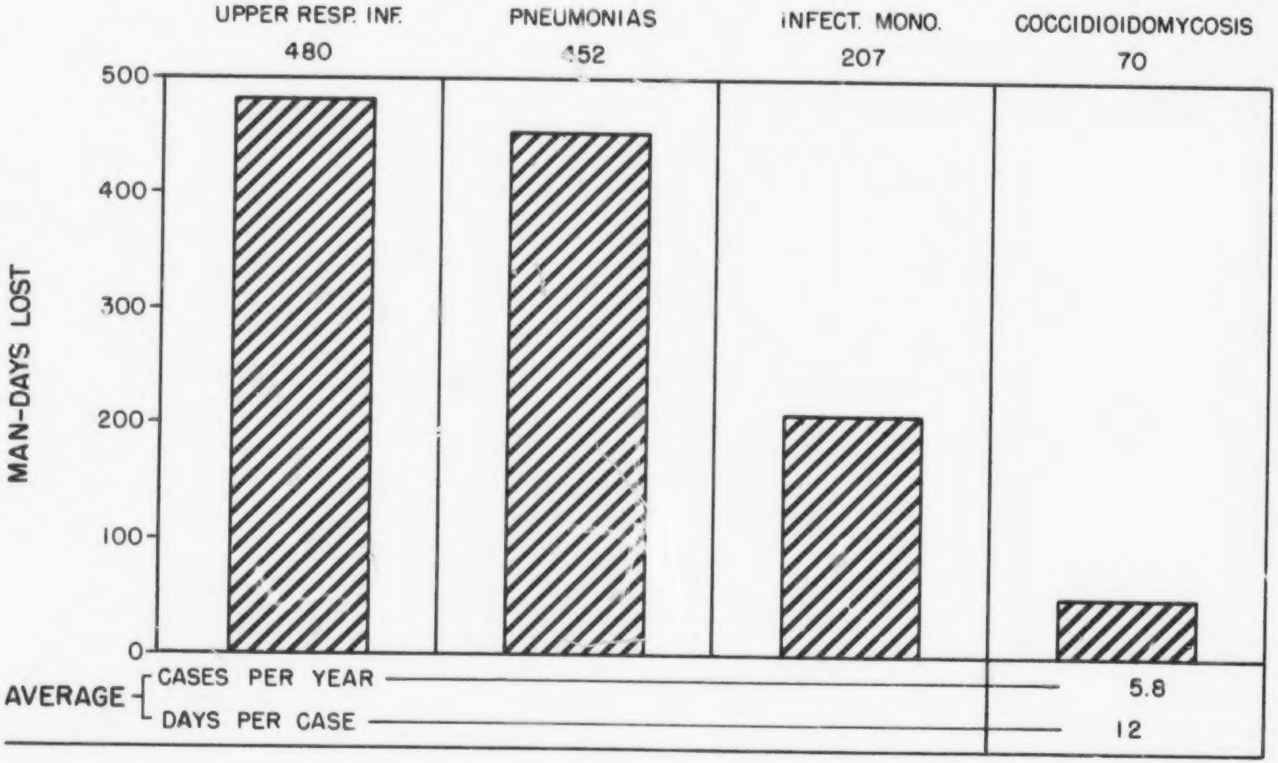


FIGURE 2

COMPARATIVE INCIDENCE OF COCCIDIOIDOMYCOSIS AND OF SELECTED DISEASES
JANUARY 1, 1954 - DECEMBER 31, 1956

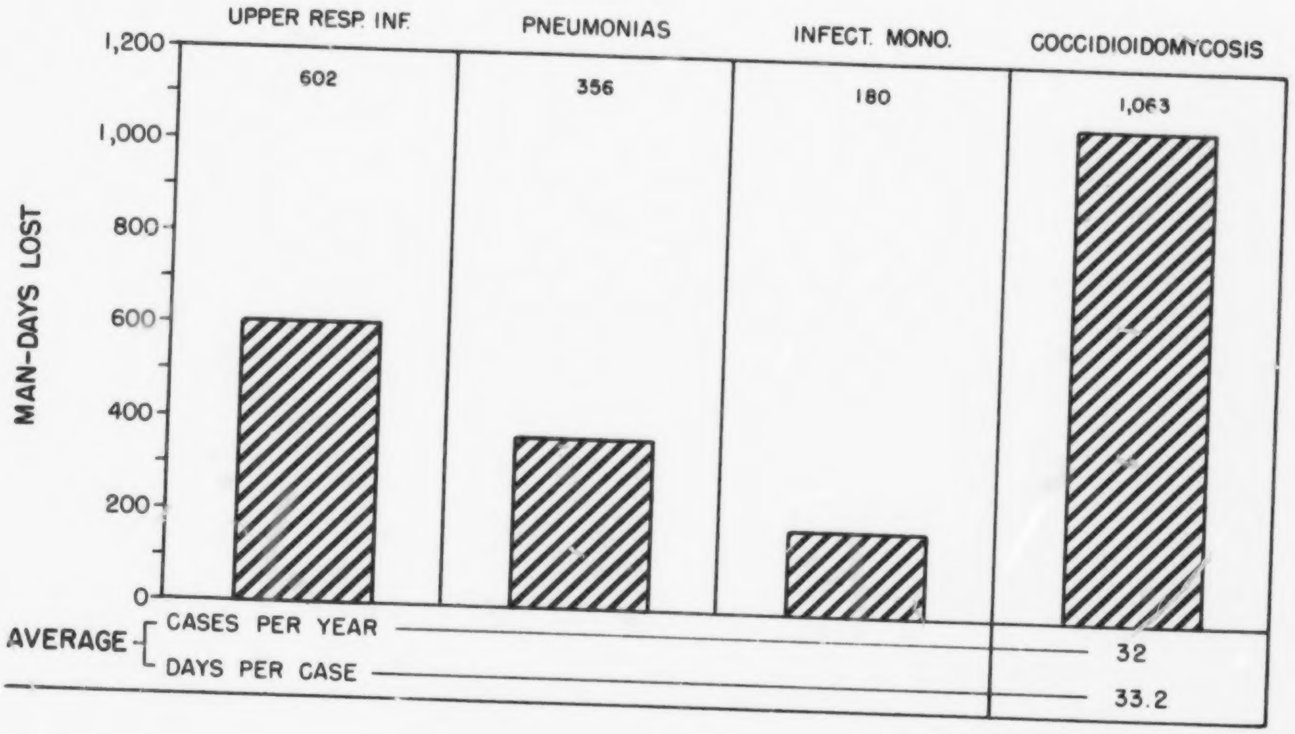
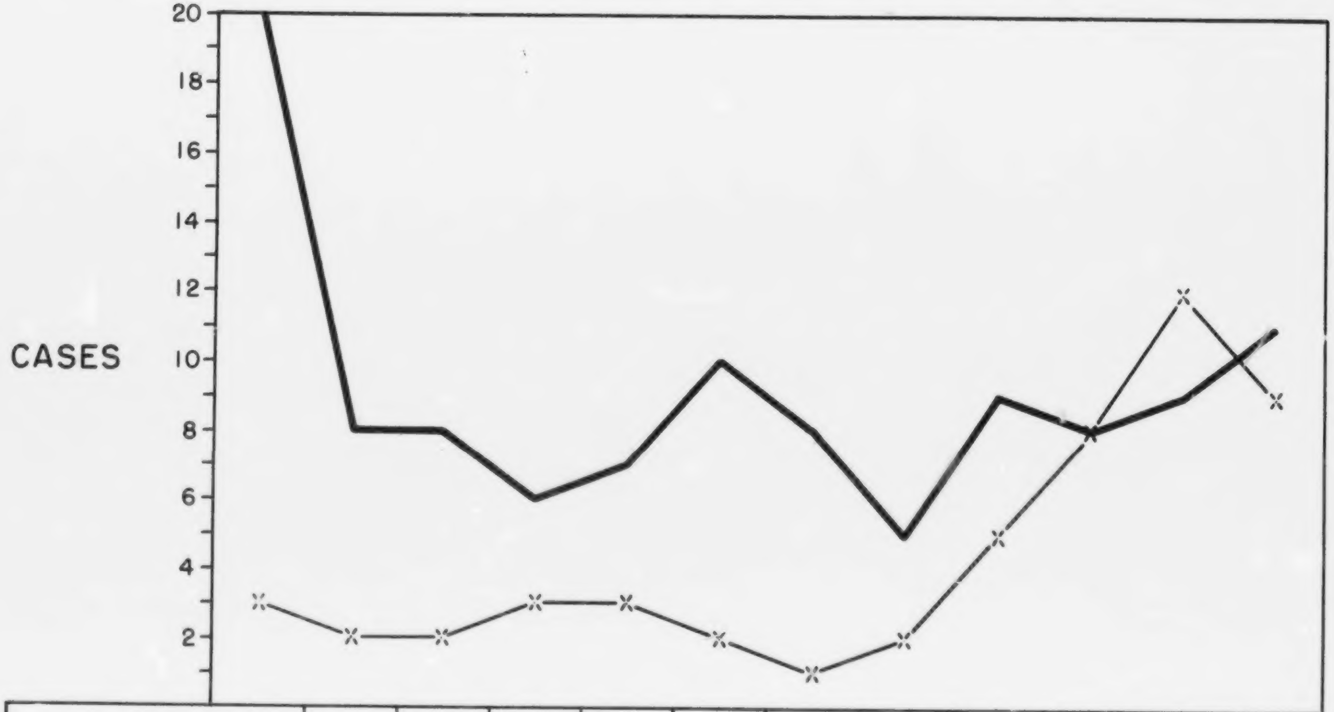


FIGURE 3

SEASONAL FLUCTUATION OF COCCIDIOIDOMYCOSIS, MAY 1951 - JANUARY 1957



| | JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| COCCI —— | 20 | 8 | 8 | 6 | 7 | 10 | 8 | 5 | 9 | 8 | 9 | 11 |
| UNCL. PNEUMONIAS × | 3 | 2 | 2 | 3 | 3 | 2 | 1 | 2 | 5 | 8 | 12 | 9 |

# Climate and Coccidioidomycosis

Paul G. Hugenholtz, M.D.

During the past few years observations to determine what influence, if any, climatic factors exerted on the rate of *Coccidioides immitis* infections among base personnel have been carried out at the Williams Air Force Base.

Data collected by several investigators have indicated that there is a correlation between rainfall, temperature, and the incidence of coccidioidomycosis. Smith *et al.*(1) demonstrated a linear relationship between seasonal rainfall and the rate of *C. immitis* infections in the San Joaquin Valley. The rate was low during the rainy season and high during the dry, dusty period that followed a rainy winter.

Soil studies carried out by Egeberg and Ely(2) revealed that a higher percentage of specimens collected at the end of the rainy season yielded *C. immitis* than those gathered during the dry season. These investigators advanced the hypothesis that the scorching heat of summer created a thin layer of virtually sterile soil in which *C. immitis* flourished after the seasonal rains provided the necessary moisture for its growth.

Pertinent to this hypothesis are the laboratory studies of Lubarsky and Plunkett.(3) They found that *C. immitis* grew better in sterilized soil than in nonsterilized soil.

Recently Maddy(4) pointed out that the coccidioidomycosis endemic areas in the United States coincide to a remarkable degree with the geographic limits of the Lower Sonoran Life Zone. He speculates that *C. immitis* grows and survives in nature only in areas with the climatic characteristics of this Zone. This Zone has average summer temperatures of 80°-90° F. with peaks of 110° F. registered in mid-afternoon that may rise even higher in the open desert. Average winter temperatures range from 50°-55° F. Rain is sparse. The yearly rainfall is 6 to 10 inches.

Williams Air Force Base is situated in the Lower Sonoran Life Zone. The climatological conditions at the Base are these: Precipitation is concentrated in two periods: (a) the winter months of December to February, with occasional extension into March and April, (b) the months of July and August, with extension into September at times. During the past 12 years annual rainfall has ranged from 2.6 to 11.6 inches. The prevailing winds occur during the spring months of March to May and during the summer months of August and September. The surface wind rose in figure 1 summarizes the wind distribution at the Base during the period of March 1942 to December 1952. The stronger winds originated in the vicinity of the Base and came from all directions. They usually had a circular track and did not cover a radius of more than 10 to 20 miles. They make up only 4.5 percent of all the winds at the Base. The most common type, 67 percent of the total, has speeds of 1.5 to 12.5 miles per hour. These winds cause localized dust storms which may reduce visibility to less than six miles. Most of these winds came from the south-southeast, southeast, and the east-southeast. This is considered to be of significance because Florence, Arizona, lies in a southeasterly direction from the Williams Air Force Base and data from the World War II Prisoner of War Camp at Florence revealed that the highest recorded *C. immitis* infection rate (50 percent) occurred there. Maddy's(4) skin test studies on the Florence prison cattle herd reveals a similar infection rate. Maddy believes that the conditions for the growth of *C. immitis*, although favorable throughout the southeastern areas of Arizona, are most favorable in the vicinity of Florence. It may be speculated that winds coming from that region carry *C. immitis* spore-laden air to the Williams Air Force Base area.

In figure 2 the possible climatological factors that influence the incidence of *C. immitis* infections are summarized. *C. immitis* is depicted as a fungus that can exist in areas with seasons of high temperature and low rainfall. During the hot, dry season the surface soil is sterilized to a certain degree. In the rainy season that follows,

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*From the U. S. Air Force Hospital, Williams Air Base, Arizona, now Boston City Hospital, Boston, Massachusetts.*

FIGURE 1

WILLIAMS AIR FORCE BASE ANNUAL
SURFACE WIND ROSE
(PERCENTAGE FREQUENCY)

BASED ON DATA: MAR 42 — DEC 52

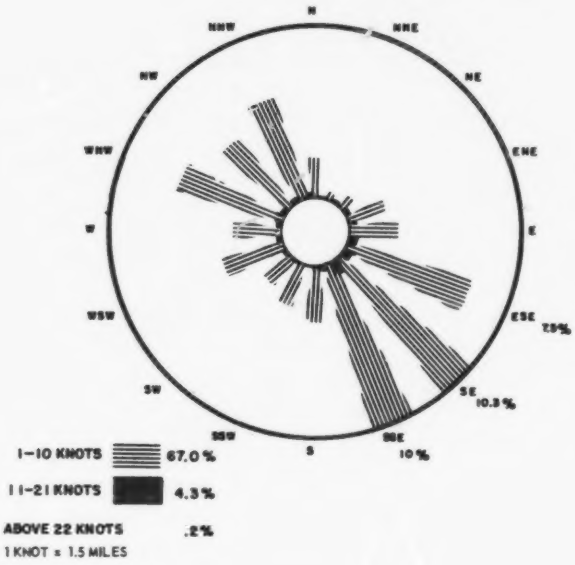
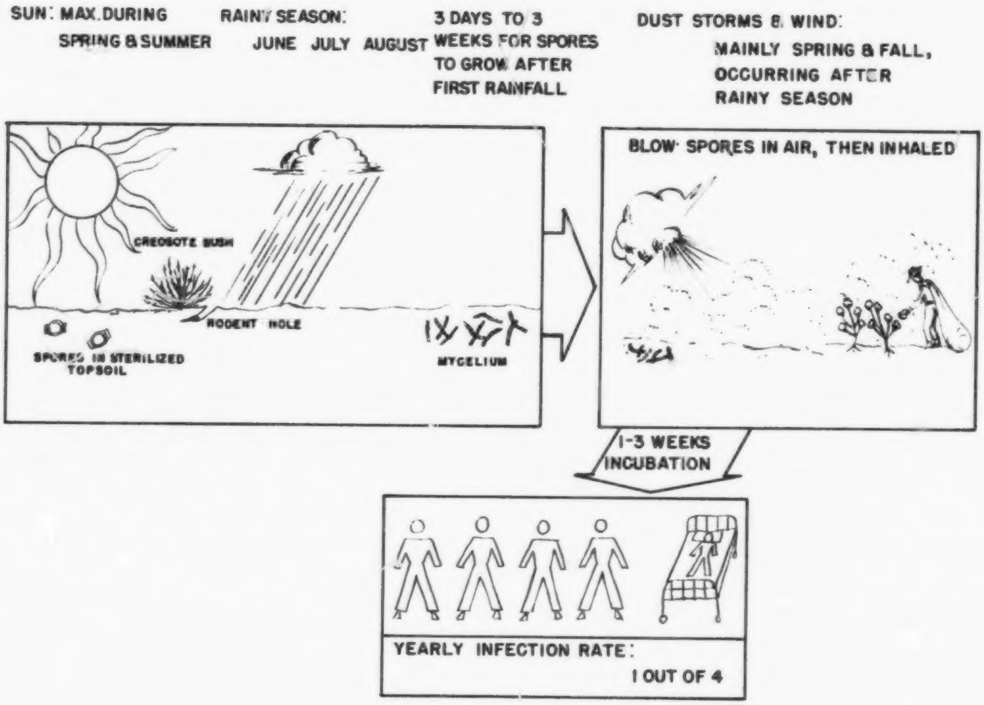


FIGURE 2


SUN: MAX. DURING SPRING & SUMMER
RAINY SEASON: JUNE JULY AUGUST
3 DAYS TO 3 WEEKS FOR SPORES TO GROW AFTER FIRST RAINFALL
DUST STORMS & WIND: MAINLY SPRING & FALL, OCCURRING AFTER RAINY SEASON
CREOSOTE BUSH
RODENT HOLE
SPORES IN STERILIZED TOPSOIL
MYCELIUM
BLOW SPORES IN AIR, THEN INHALED
1-3 WEEKS INCUBATION
YEARLY INFECTION RATE: 1 OUT OF 4

*C. immitis*, which survived the hot, dry period in the deeper strata of the soil, resumes growth and migrates to the surface soil. There it produces abundant arthrospores, which are picked up by the prevailing winds and scattered throughout the region, causing infection through inhalation.(5)

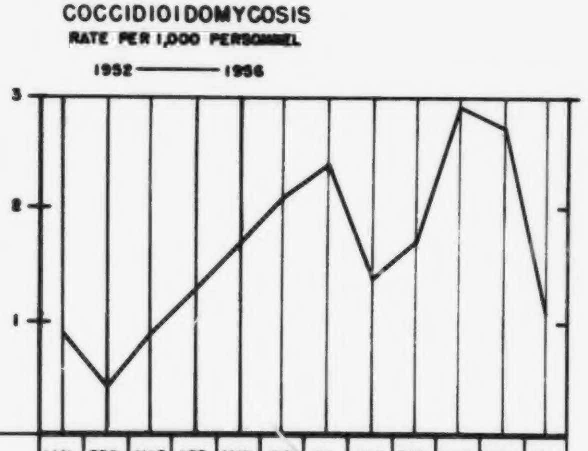
In an effort to check this hypothesis, an analysis was made of the coccidioidomycosis cases that had occurred at the Williams Air Force Base during the past few years. It was found that there were two seasonal peaks in hospital admissions during 1952-56. The first peak occurred in July and the second during the months of October and November (fig. 3). Analysis of admissions for the 1942-55 period showed a correlation between the monthly admission rate and monthly precipitation (fig. 4). It will be noted that the peak number of cases occurred during the months with the lowest rainfall (June, July and October, November).

Admittedly, there are too many variables in this phenomenon to draw any definite conclusions. However, it is interesting to note that Arizona Health Department records reveal similar peaks during the past 10 years in Maricopa* and Pima counties.

On the basis of these findings, one could predict that there would be a relatively low rate of *C. immitis* infection in spring and summer if rains were absent or below normal during the usual wet season. A dry winter should be followed by a relatively low infection rate in the spring and summer. A dry July and August should be followed by a low rate of infection in the fall.

Infections during 1955-56 at the Base followed such a predicted course. Weather was extremely dry at that time. The Base hospital, which usually has approximately 60 clinically active cases of coccidioidomycosis, had only 18 cases during that entire year. However, five active cases occurred in October following a dust storm that developed after a short period of rain. The effect of the unusually dry weather of that year on *C. immitis* infection is further illustrated by skin test observations. In 1956 skin tests were carried out on the Base personnel at three-month intervals. As seen in figure 5, there were twice as many conversions from negative to positive during the last trimester of the year than during the two previous ones.

FIGURE 3



| MONTHS | JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TOTAL NUMBER CASES 1952—1956 | 14 | 7 | 14 | 20 | 26 | 33 | 36 | 22 | 27 | 44 | 41 | 14 |
| AVERAGE NUMBER PER MONTH | 2.8 | 1.4 | 2.8 | 4 | 5.2 | 6.6 | 7.2 | 4.4 | 5.4 | 8.8 | 8.2 | 3.5 |
| RATE PER 1,000 | .9 | .4 | .9 | 1.3 | 1.7 | 2.1 | 2.4 | 1.4 | 1.7 | 2.9 | 2.7 | 1.1 |

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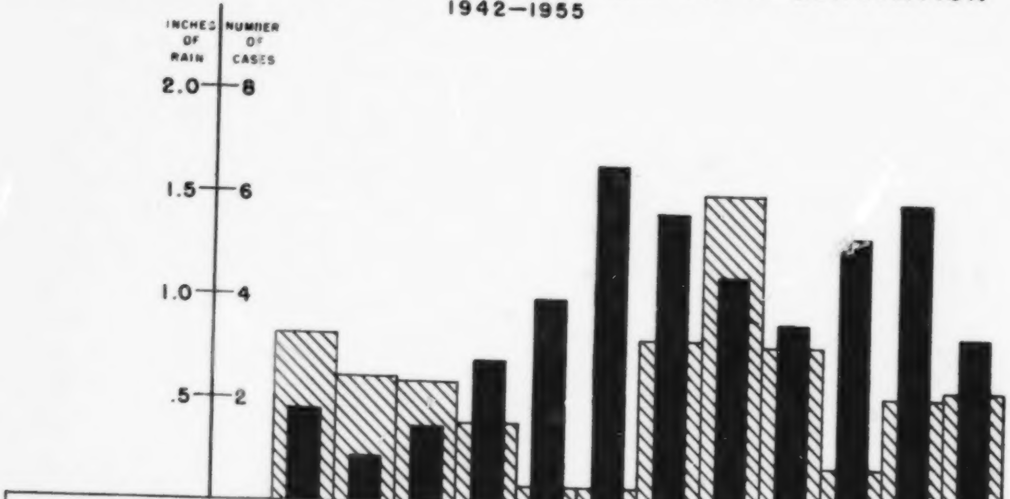
*The Williams Air Force Base is located in this county.

FIGURE 4

## RELATIONSHIP OF COCCIDIOIDAL INFECTIONS AND PRECIPITATION

1942—1955



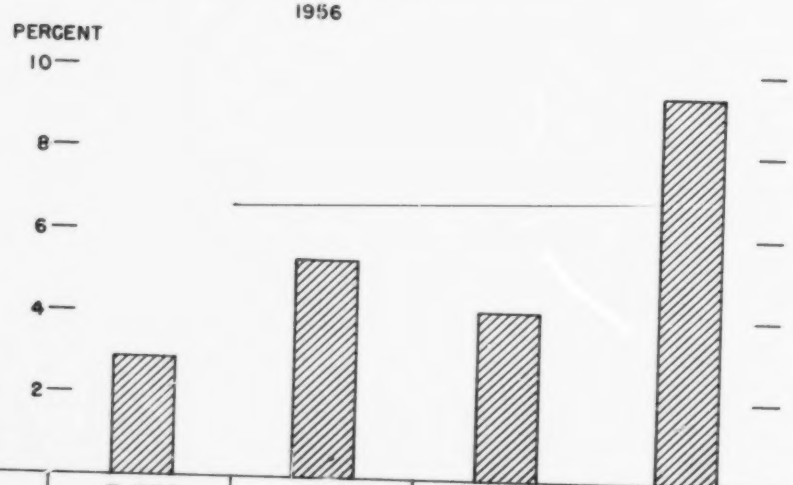
| MONTHS | | JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AVERAGE NUMBER OF INFECTIONS | ■ | 1.8 | .9 | 1.5 | 2.8 | 4.0 | 6.6 | 5.7 | 4.5 | 3.6 | 5.3 | 6.0 | 3.4 |
| AVERAGE INCHES OF RAINFALL | ▨ | .82 | .61 | .59 | .39 | .09 | .09 | .81 | 1.53 | .79 | .21 | .55 | .59 |

FIGURE 5

## COCCIDIOIDIN SKIN TEST RESULTS

PERCENT POSITIVES

1956



| THREE MONTH PERIODS ENDING | 7 FEB | 7 MAY | 14 AUG | 7 NOV |
|---|---|---|---|---|
| NUMBER TESTED | 291 | 205 | 192 | 159 |
| NUMBER POSITIVE | 10 | 11 | 8 | 15 |
| PERCENT POSITIVE | 2.9 | 5.3 | 4.1 | 9.4 |

To further evaluate this hypothesis, a detailed analysis was made of coccidioidomycosis admissions at the Base for the past 14 years and of the meteorological conditions that prevailed during that time. These data are summarized in table 1.

A low and a high peak of infection are evident. From December to mid-April relatively few cases were diagnosed. From the latter half of April through November the number increased. The large number of cases reported in the last two weeks of November, after a decline from a mid-summer high, is of even greater interest. While this increase might be attributed to a severe outbreak during a single year, examination of the data revealed that there were 15 cases for the same period in 1945, 6 in 1947, 7 in 1951, and 12 cases in 1953. There were fewer than 3 cases in only 4 of the 14 years.

Rainfall in this area is concentrated during the summer months. Practically no rain falls during May and June. Dust storm peaks occur in spring, associated with the general rise of heated desert air and a flow of cooler air from higher elevations, and in summer, generally associated with thunderstorm activity. In the fall there are few dust storms and rainfall is fairly light to moderate.

In considering the gross relationship between the number of clinical cases and environmental factors, linear correlation coefficients were computed. (Some nonlinear relationships may be present but were not investigated in the available

**TABLE 1**
**SEMI-MONTHLY DISTRIBUTION OF COCCIDIOIDOMYCOSIS CASES AND SELECTED ENVIRONMENTAL FACTORS**

| | | Averages: January 1943 – December 1956 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total No. Cases | Total Rain-fall | No. Rainy Days | No. Days > Trace | No. Days > .10 | No. Days With Dust Storms | No. Hrs. Dust Storms | Average Temperature |
| Jan. | 1st half | 19 | 4.15 | 31 | 24 | 15 | 2 | 3 | 50.6 |
| | 2nd half | 15 | 7.25 | 44 | 28 | 19 | 9 | 10 | |
| | | 34 | 11.40 | 75 | 52 | 34 | 11 | 13 | |
| Feb. | 1st half | 14 | 2.99 | 33 | 19 | 10 | 4 | 15 | 54.3 |
| | 2nd half | 16 | 5.09 | 35 | 24 | 13 | 6 | 31 | |
| | | 30 | 8.08 | 68 | 43 | 23 | 10 | 46 | |
| March | 1st half | 19 | 4.22 | 33 | 17 | 11 | 9 | 34 | 58.6 |
| | 2nd half | 18 | 2.88 | 26 | 13 | 6 | 14 | 63 | |
| | | 37 | 7.10 | 59 | 30 | 17 | 23 | 97 | |
| April | 1st half | 18 | 2.02 | 20 | 9 | 6 | 11 | 54 | 68.2 |
| | 2nd half | 43 | 2.39 | 26 | 16 | 6 | 16 | 44 | |
| | | 61 | 4.41 | 46 | 25 | 12 | 27 | 98 | |
| May | 1st half | 32 | .90 | 13 | 5 | 2 | 13 | 20 | 76.2 |
| | 2nd half | 39 | .17 | 10 | 1 | 1 | 18 | 34 | |
| | | 71 | 1.07 | 23 | 6 | 3 | 31 | 54 | |
| June | 1st half | 35 | .45 | 9 | 3 | 2 | 6 | 14 | 83.7 |
| | 2nd half | 57 | .70 | 11 | 5 | 2 | 15 | 29 | |
| | | 92 | 1.17 | 20 | 8 | 4 | 21 | 43 | |
| July | 1st half | 42 | 2.11 | 49 | 19 | 8 | 26 | 39 | 90.0 |
| | 2nd half | 58 | 9.30 | 87 | 43 | 20 | 45 | 65 | |
| | | 100 | 11.41 | 136 | 62 | 28 | 71 | 104 | |
| Aug. | 1st half | 51 | 9.33 | 69 | 46 | 25 | 27 | 35 | 88.2 |
| | 2nd half | 38 | 9.36 | 50 | 29 | 13 | 27 | 38 | |
| | | 89 | 18.69 | 119 | 75 | 38 | 54 | 73 | |
| Sept. | 1st half | 27 | 1.01 | 19 | 11 | 3 | 16 | 24 | 83.6 |
| | 2nd half | 44 | 10.17 | 30 | 21 | 15 | 12 | 20 | |
| | | 71 | 11.18 | 49 | 32 | 18 | 28 | 44 | |
| Oct. | 1st half | 40 | 1.48 | 27 | 10 | 5 | 4 | 12 | 71.5 |
| | 2nd half | 44 | 1.07 | 27 | 16 | 6 | 8 | 34 | |
| | | 84 | 2.55 | 54 | 26 | 11 | 12 | 46 | |
| Nov. | 1st half | 39 | 3.07 | 23 | 11 | 9 | 6 | 10 | 59.6 |
| | 2nd half | 65 | 4.79 | 30 | 20 | 10 | 4 | 14 | |
| | | 104 | 7.86 | 53 | 31 | 19 | 10 | 24 | |
| Dec. | 1st half | 28 | 3.02 | 25 | 18 | 11 | 4 | 5 | 51.7 |
| | 2nd half | 14 | 4.66 | 53 | 21 | 11 | 5 | 13 | |
| | | 42 | 7.68 | 78 | 39 | 22 | 9 | 18 | |

time.) Correlations between the four rainfall measurements and total cases were: 0.022, 0.156, 0.127, 0.036, respectively, using the 24 semimonthly values. For the two storm variables the correlations were: 0.467 and 0.434, respectively. While none of these coefficients suggest strong relationship between these environmental factors and incidence of coccidioidomycosis, the relationship with dust storms is more significant than with rainfall. The one high value for the number of cases in the latter part of November is the principal factor which reduces the correlation coefficient. For example, removing the one value for that period raises the correlation between the number of days with dust storms of over one-hour duration and the total number of cases from 0.467 to 0.665. This suggests that if a variable could be found which explained the peak in November, a better predictive relationship might be established.

An additional correlation between monthly temperatures and the number of cases per month was computed with the surprisingly high value of 0.734. The difference between this correlation and those obtained by using the other measure is in the sharp rise in temperature in the spring and the great increase in the number of cases of coccidioidomycosis. Unfortunately, the November anomaly is not explained by the change in temperature. The correlations between the rainfall and dust storm values and number of cases were not increased preceptably by using monthly values, so the "smoothing effect" cannot be regarded as a factor.

On the basis of the total number of yearly recorded cases (table 2), several individual years might be excluded from the detailed analysis because there is too little variation throughout the year to disclose any special relationships. Data for 1943 and 1953 were examined for weather-coccidioidomycosis relationship. These data are presented in table 3.

In 1943 a large number of cases of this disease occurred between late April and mid-August, a period which had little moisture prior to August and very few dust storms until late July. There were a large number of cases throughout most of 1953, with a peak in April and another in the late fall. There appeared to be moderately favorable weather conditions in the spring, but most of the fall was dry with only a small amount of dust in October.

A subdivision of periods into classes regarding rainfall and dust was made with the following results (table 4).

### TABLE 2

### ANNUAL INCIDENCE OF CLINICAL CASES OF COCCIDIOIDOMYCOSIS 1943 – 1956*

| Year | Total Cases |
|---|---|
| 1943 | 103 |
| 1944 | 55 |
| 1945 | 81 |
| 1946 | 66 |
| 1947 | 35 |
| 1948 | 35 |
| 1949 | 29 |
| 1950 | 39 |
| 1951 | 40 |
| 1952 | 87 |
| 1953 | 136 |
| 1954 | 74 |
| 1955 | 17 |
| 1956 | 18 |

*While the number of cases was in some degree associated with average number of base personnel, the wide variation from 17 to 136 cases in 3 years is not explained easily on this basis. There appeared to be no general environmental conditions associated with these changes.

A much greater difference was noted when the presence of dust (2.70 vs. 2.15) was averaged over rain conditions rather than when rain was averaged over dust conditions (2.40 vs. 2.46). The depression of number of cases with rain, but no dust, may support the hypothesis that spore production may be inhibited by other organisms in the presence of too much moisture.

It is realized that these remarks have been largely theoretical and based on an incomplete study, but they may serve to stimulate studies by other investigators.

A unique opportunity for testing this hypothesis was presented at the Williams Air Force Base. The Base is within the coccidioidomycosis endemic area. It has a fully equipped weather station which kindly has supplied the detailed meteorologic data. We wish to extend our appreciation to

## TABLE 3

## RELATION OF WEATHER CONDITIONS TO INCIDENCE OF COCCIDIOIDOMYCOSIS

| | | 1943 | | | 1953 | | |
|---|---|---|---|---|---|---|---|
| | | Cases | Total Rainfall | Total hrs. dust | Cases | Total Rainfall | Total hrs. dust |
| Jan. | 1 | 8 | .20 | 0 | 3 | .30 | 1 |
| | 2 | 6 | .51 | 0 | 0 | 0 | 1 |
| Feb. | 1 | 2 | .10 | 0 | 2 | 0 | 4 |
| | 2 | 1 | 0 | 0 | 1 | .53 | 11 |
| March | 1 | 2 | .51 | 2 | 6 | .54 | 0 |
| | 2 | 2 | T | 0 | 3 | T | 14 |
| April | 1 | 1 | T | 4 | 4 | 0 | 0 |
| | 2 | 6 | T | 0 | 13 | .03 | 5 |
| May | 1 | 4 | T | 1 | 4 | 0 | 4 |
| | 2 | 8 | 0 | 0 | 4 | T | 8 |
| June | 1 | 6 | T | 1 | 3 | T | 6 |
| | 2 | 13 | 0 | 0 | 7 | 0 | 3 |
| July | 1 | 1 | T | 0 | 7 | .16 | 1 |
| | 2 | 9 | .16 | 4 | 9 | 2.04 | 4 |
| Aug. | 1 | 10 | .82 | 4 | 1 | .06 | 3 |
| | 2 | 4 | .28 | 2 | 6 | .15 | 3 |
| Sept. | 1 | 4 | 0 | 1 | 6 | 0 | 0 |
| | 2 | 3 | 2.06 | 1 | 3 | 0 | 0 |
| Oct. | 1 | 3 | T | 0 | 15 | T | 7 |
| | 2 | 0 | .14 | 5 | 13 | T | 3 |
| Nov. | 1 | 2 | 0 | 0 | 6 | .19 | 1 |
| | 2 | 3 | 0 | 1 | 12 | .16 | 0 |
| Dec. | 1 | 1 | .43 | 0 | 4 | T | 0 |
| | 2 | 4 | .60 | 1 | 4 | T | 0 |

## TABLE 4

## AVERAGE NUMBER OF CASES PER TWO-WEEK PERIOD

| No Measurable Rain | Measurable Rain* | Average |
|---|---|---|
| 2.33 | 1.95 | 2.15 |
| 2.48 | 2.86 | 2.70 |
| 2.40 | 2.46 | 2.43 |

*Rainfall of > .01.

Dr. Tucker, statistician at the Agricultural Experimental Station of the University of Arizona (Tucson), who computed the statistical data.

## Summary

Analysis of coccidioidomycosis admissions and climate data at the Williams Air Force Base appears to indicate some relationship between weather conditions and the incidence of coccidioidomycosis. Rainfall, dust, and temperature are factors of particular importance. This relationship varies within the endemic area according to local climatological conditions. A hypothesis of such a relationship has been presented for the Williams Air Force Base. The essentials of this hypothesis are: (1) *C. immitis* spores are permanent components of the soil of the endemic zone; (2) periodic seasonal changes in soil moisture and temperature favor the growth of *C. immitis* in soil; (3) an increase in soil moisture within a period of three days to several weeks prior to dust storms stimulates arthrospore formations; (4) if these factors follow each other in a certain time relationship, a rise in disease incidence can be predicted.

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# Ecological Factors Possibly Relating to the Geographic Distribution of *Coccidioides immitis*

Keith T. Maddy, D.V.M., M.P.H.

A number of interesting studies have shown how the ecological requirements of various microorganisms affect the geographic distribution of certain animal and human diseases.

Study of the relationship of soil types in midwestern United States to the distribution of swine erysipelas indicated that *Erysipelothrix rhusiopathiae* was likely to become established in the soil in that area(1) only when the soil became slightly alkaline. Other investigators(2) showed that soils of the United States that were neutral or slightly alkaline, rich in nitrogen, moist and poorly drained were the ones in which *Bacillus anthracis* was most likely to become established.

Zeidberg(3) has offered data that purported to show a correlation between the existence of a significant number of histoplasmin-reacting persons and the occurrence of red-yellow podzolic soils in their locale. Zeidberg and Ajello(4) discovered a correlation between *Histoplasma capsulatum* and chicken habitats. It has been observed that soils from which *H. capsulatum* has been isolated had a higher acidity than negative soils.(5) They point out that although in the laboratory *H. capsulatum* is capable of abundant growth over a wide pH range, perhaps under natural conditions, the pH level is more important. Furcolow and Horr(6) found that the area of the United States where the average annual precipitation is from 35 to 50 inches and the average summer temperature is between 70° F. and 80° F. is in the Middle Central and Middle Eastern States where there is a high level of histoplasmin sensitivity. In their opinion the direction of the prevailing winds over this area tends to limit the fungus to the eastern and northeastern parts of the United States. The area between the Appalachians and the Atlantic coastline is only mildly endemic, although temperatures are within Furcolow and Horr's selected ranges. This area is protected by mountains from the main endemic area, and the prevailing winds of the coastal area blow in a large circle over it.

Plant pathologists have studied the relationship of climate to certain fungal infections in plants. Some of these studies have progressed to the point where epidemics of these infections can be predicted from weather observations.(7-17) The possible significance of such factors in regard to histoplasmosis has been shown.(6)

Smith(18) mapped out the areas where human infections with *Coccidioides immitis* have occurred in the United States (fig. 1). The close correlation of this area with the Lower Sonoran Life Zone of the United States should be noted (fig. 2).

## Observations

In 1898, Merriam(19) classified the United States into several biological life zones. One of these is the Lower Sonoran Life Zone. Since that time there have been numerous modifications and refinements of the life-zone concept. These zones were first divided according to the sums of the degrees of temperature above a given temperature for a year, added to the mean temperature for the hottest six weeks. This classification was based on the knowledge that plants grow only when the environmental temperature is above a certain point. The zones were then subdivided into humid and arid provinces and the distribution of plant and animal life within the zones was studied. In broad geographic areas, these life zones are useful in the ecological study of plant and animal life. In small geographic subdivisions, study of plant and animal associations is more useful in analyzing the ecology of the area.(20) Slope exposure, air currents, streams carrying cold water, evaporation from moist soil, proximity to large bodies of water, influence of lingering snow banks and of glaciers, changes in vegetative covering, extent of a mountain area, rock surfaces, and other miscellaneous factors can alter the characteristics of a life zone in a local area.

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*From the Communicable Disease Center, assigned Arizona State Department of Health, Phoenix.*

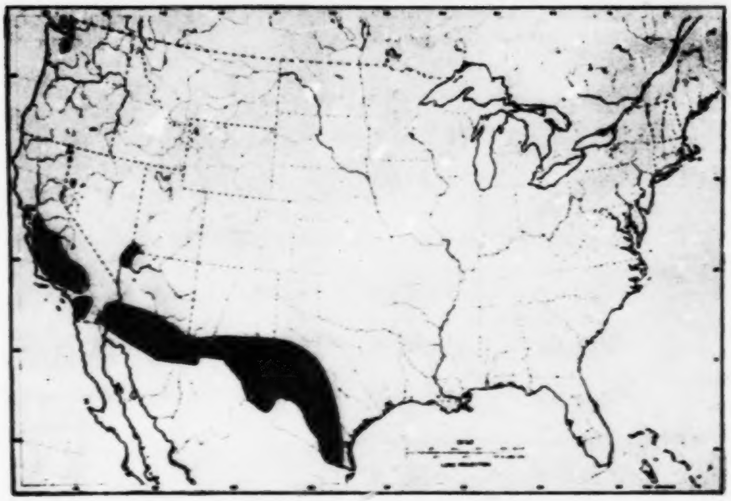

Figure 1. Endemic coccidioidomycosis areas in the United States as designated by Smith in 1951.(18) *(Reproduction permission granted by California Medicine.)*

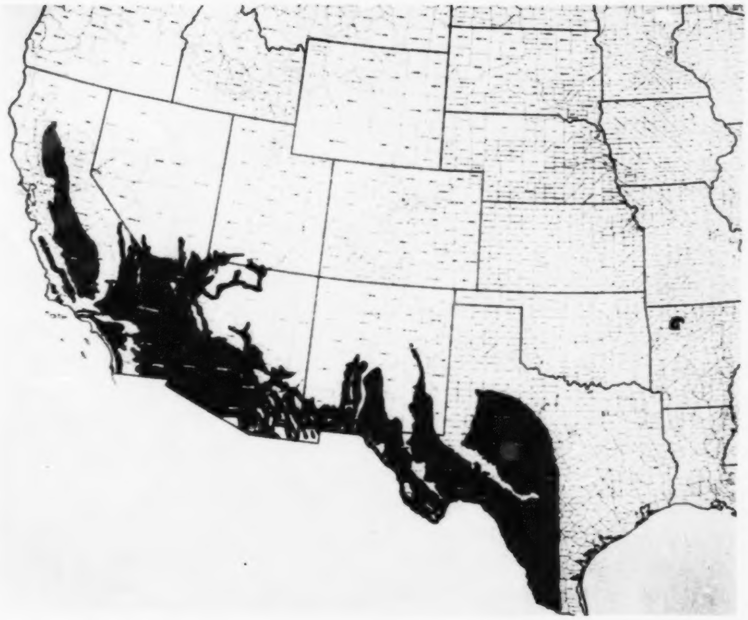

Figure 2. Lower Sonoran Life Zone in the United States.

The life-zone concept is empirical and, as such, biologists do not agree on the number of zones or where to divide them. As an example, at the eastern end of the Lower Sonoran Life Zone, there is a question about the exclusion of the Edwards Plateau in Texas and parts of northwest Texas and southwest Oklahoma. Biologists do not agree on the value of the life-zone concept in the study of plant and animal life; some prefer to use a biotic-province concept for such studies.

The Lower Sonoran Life Zone is characterized by arid and semiarid climates, with hot summers and few winter freezes, low altitude, and alkaline soil. Plants and animals are limited to those types that can survive in this environment. This environment, and only this environment, seems to permit *C. immitis* to exist in nature as a soil organism.

It is postulated that a careful study of the characteristics of the Lower Sonoran Life Zone would help to understand the epidemiology of coccidioidomycosis. Such a study has been undertaken by the author.

## Arizona Studies

This investigation began with the observation that the creosote bush, *Larrea tridentata* (fig. 3), grows in all parts of Arizona where coccidioidomycosis is known to be endemic. It was observed that the bush does not grow in any of the areas believed to be nonendemic. An opportunity was presented to skin test dairy cattle with coccidioidin in the Verde Valley. This valley, which lies about 100 miles north of Phoenix, was believed to be out of the coccidioidomycosis area. However, the creosote bush was found growing there in abundance, and it was predicted that *C. immitis* would be found in the area. Tests proved that a large percentage of the home-raised dairy cattle were coccidioidin positive.

Although Bullhead City, Kingman, and Wikieup, in northwestern Arizona, are out of the known endemic area, they are in the creosote bush locale(21,22,23) (figs. 4 and 5) and coccidioidin tests on the cattle revealed infection with the fungus. Fewer infections were found at the higher elevations. Just south of Kingman, in the Hualpai Mountains, above the creosote bush terrain in the Upper Sonoran Life Zone, a herd of cattle was tested. No evidence of infection was found in the home-raised animals. Some animals brought there from Kingman, and from a known endemic area northeast of San Diego, California, were coccidioidin positive.

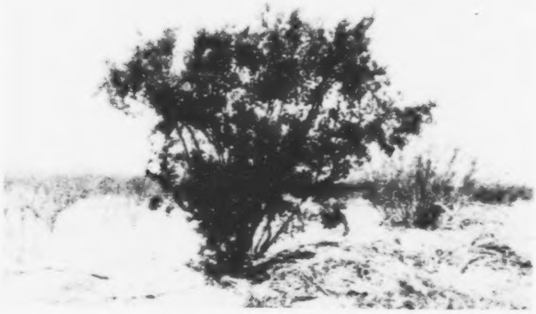

Figure 3. The creosote bush, (*Larrea tridentata*).

About this time it was learned that the creosote bush was one of the chief indicator plants used in designating the Lower Sonoran Life Zone, with the exception of some areas in west Texas and the San Joaquin and Sacramento Valleys of California which receive more rainfall and gradually blend into other life zones. The life-zone maps when correlated with altitude and rainfall data(24,25) have been found effective in predicting endemic areas.

The map of the Lower Sonoran Life Zone in Arizona (fig. 6)(26) shows that an endemic area should exist along the Colorado River across northern Arizona to the Utah line. Future projects will include some skin tests of persons and animals in these areas. Mules that live in the Transition Life Zone at the south rim of the Grand Canyon carry tourists down through the Upper Sonoran Life Zone onto the floor of the Canyon in the Lower Sonoran Life Zone. A check on the correlation of the life zones and endemic areas might be made through tests on these mules. Also tests on persons and animals on the Havasupai Indian Reservation down in the Grand Canyon might provide valuable data.

Studies carried out on the Indian reservations of Arizona(27) have revealed a high percentage of skin test positive persons on the Papago, Pima, and San Carlos Reservations. These reservations in south central Arizona are at low altitudes. At Fort Apache – a much higher elevation – almost no infection was detected by skin tests. Studies by the United States Army Air Corps, the Army Epidemiological Board, and the United States Air Force revealed that Luke Air Force Base near Phoenix, Williams Air Force Base near Chandler, Gila Bend Air Base near Gila Bend, and Marana

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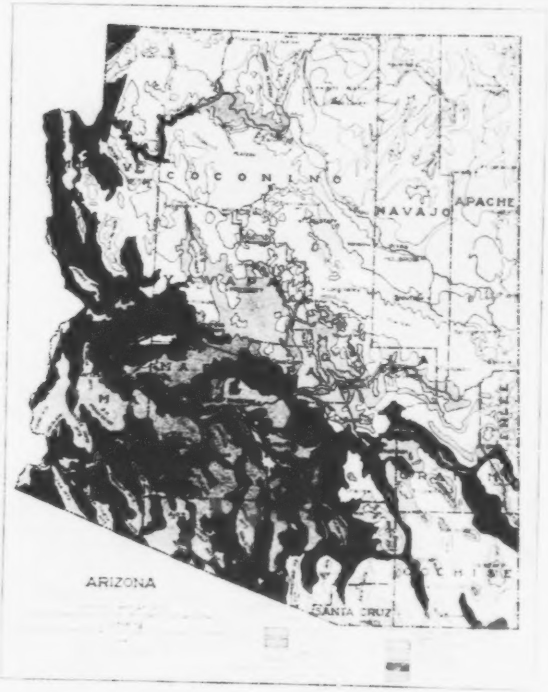

Figure 4. Map of the natural vegetation of Arizona. (The dark areas of the southwestern parts of the State are the creosote bush areas.) *(Reproduction permission granted by University of Arizona.)*

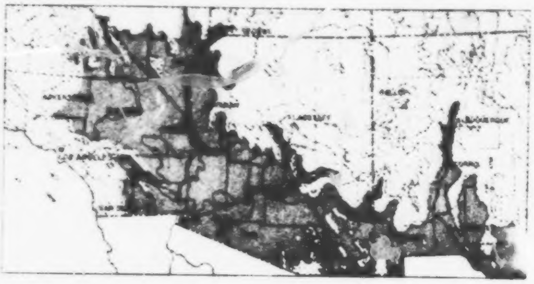

Figure 5. Distribution map of the creosote bush *(Larrea tridentata)* over part of the southwestern deserts of the United States. *(Reproduction permission granted by University of Arizona.)*

and Davis-Monthan Air Force Bases near Tucson were in endemic areas.(28,29) A World War II prisoner-of-war compound at Florence was found to be the most infective area known. Fifty percent of the susceptibles in this compound converted to a positive skin test within six months.(30)

Studies on school children in the Phoenix area revealed a high number of skin-test positive individuals.(31,32)

Observations on infections acquired by feedlot cattle(33,34) have shown various areas of south central Arizona to be endemic. Skin tests are being made on a number of home-raised cattle throughout Arizona. Preliminary results show that in areas known to be infective for man there is also infectiveness for cattle. *C. immitis* is being found in new areas and the relative infectivity of the various areas is being determined.

Studies made during and after World War II(35) reveal no endemicity at Fort Huachuca in the extreme southern part of the State. This fort is located just above the Lower Sonoran Life Zone at about 5,000 feet altitude.

The extreme western, southeastern, and the northwestern parts of Arizona within the Lower Sonoran Life Zone seem to have low infectivity levels. Apparently the south central part of the State is the most infective coccidioidomycosis

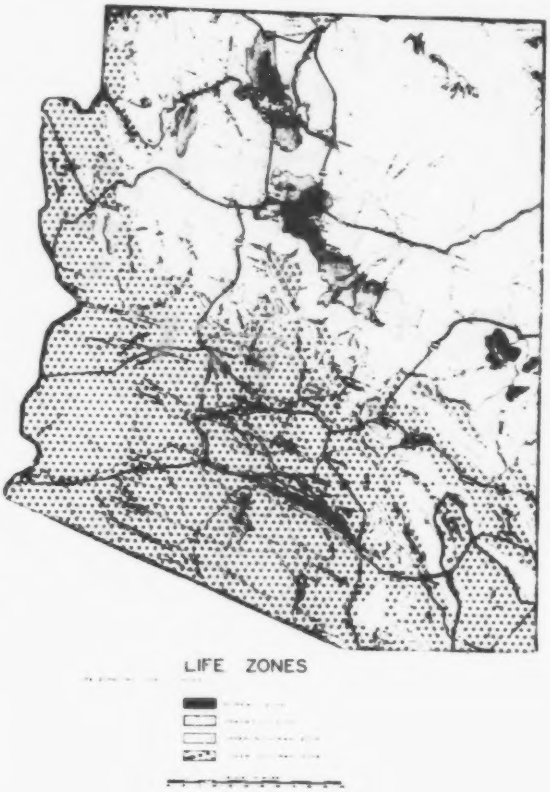

Figure 6. Map showing the Lower Sonoran Life Zone in Arizona. *(Reproduction permission granted by U. S. Department of Interior.)*

area for mammals in the world. In attempting to determine the factors that favor the natural propagation of the fungus, climatic characteristics in south central Arizona were noted. The mean July temperature in the shade is around 90° F.; the July mean maximum temperature is around 10[illegible]° F. The mean January temperature is around 50° F.; rarely does a freezing temperature occur at the ground level for more than a few hours. The rainfall averages about 9 inches per year and it falls mostly in 2 seasons – summer and winter. The mean relative humidity at 5:30 a.m. is about 59 percent in July and about 82 percent in January. The mean relative humidity at 5:30 p.m. is about 30 percent in July and about 51 percent in January. The soil is mildly alkaline in reaction. The vegetation is quite typical of the Lower Sonoran Life Zone but it is somewhat more abundant than in other areas of the Zone as the rainfall is above average.

### New Mexico Studies

A look at the Lower Sonoran Life Zone in New Mexico[36] (fig. 7) shows that infections could be expected to occur at such places as Lordsburg, Deming, Las Cruces, Hot Springs, Socorro, Alamogordo, Roswell, and Carlsbad. Santa Fe and Albuquerque are out of the Lower Sonoran Life Zone and thus the creosote bush zone. Since Albuquerque is in the desert, it has been expected that coccidioidomycosis might be acquired there, but no cases have been diagnosed. The minimum winter temperatures in Santa Fe and Albuquerque are close to, or are possibly just below, the minimum temperatures at which *C. immitis* can exist in nature.

### Texas Studies

In Texas the creosote bush belt[37] (fig. 8) extends down along the Rio Grande beginning in a 180-mile-wide belt at the south New Mexico line. It tapers as it follows the river southward and ends just south of Laredo. The endemic areas in Texas are within this belt. The eastern portion of the Lower Sonoran Life Zone in Texas ends where rainfall increases. Although most of eastern Texas and most of the southeastern United States have just as many units of high temperature as the Lower Sonoran Life Zone, rainfall is significantly higher. This area belongs in the Lower Austral Life Zone. The Upper Austral Life Zone just to the north is almost identical to the endemic area for histoplasmosis. Indicator plant and animal species have

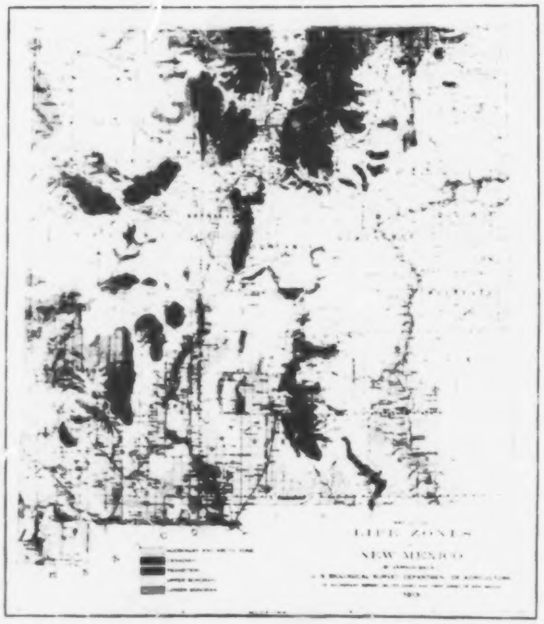

Figure 7. Map of the life zones of New Mexico. (The Lower Sonoran Life Zone is designated in gray – lower half of the State.) *(Reproduction permission granted by U. S. Department of Agriculture.)*

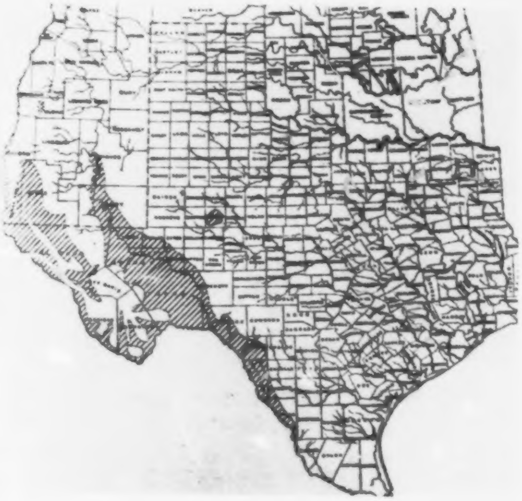

Figure 8. Distribution of creosote bush (*Larrea tridentata*) in Texas and parts of New Mexico. *(Reproduction permission granted by U. S. Department of Agriculture.)*

been selected for the Lower Sonoran Life Zone in Texas[37,38,39] (fig. 9). The eastern limit of the Lower Sonoran Life Zone is almost identical with the extent of the coccidioidomycosis endemic area.[18] The limiting factor for the flora and fauna at the eastern edge of the zone is the increased rainfall that permits other species of plants, fungi, and animals to thrive better, thus crowding them out.

### Utah Studies

In Utah, the St. George region in the southwestern corner is known to be mildly infective. Tests on school children[40] and cattle skin tests[41] reveal the infective area to be almost identical with the Lower Sonoran Life Zone there (fig. 10). Tests have not been made in the Lee's Ferry area. Although there are other arid areas farther north, the two places mentioned are the only ones in Utah where the minimal winter temperatures stay high enough to permit the specialized flora and fauna characteristic of the Lower Sonoran Life Zone to exist.

### Nevada Studies

Some human cases have been observed in the Las Vegas area of Nevada. Skin tests on dairy cattle revealed the Logandale-Overton area (60 miles north of Las Vegas) to be endemic. The Lower Sonoran Life Zone in Nevada (fig. 11)[42] is roughly below a line from St. George, Utah, to Scottie's Castle in California's Death Valley. The Zone reaches north into the Nevada valleys almost to Caliente and Alamo, although the upper areas of the Mormon, Sheep, and Charleston Mountains are out of the Zone. The arid area continues northward but the minimum temperatures in winter restrict the flora and fauna typical of the Lower Sonoran Life Zone to the southern tip of the State.

### California Studies

In California the entire Mohave and Sonoran Deserts are within the Lower Sonoran Life Zone[43] (fig. 12). Since relatively few people live in these desert areas, few coccidioidomycosis studies on humans have been possible. Not all of these two areas were included[18] in the areas of proved endemicity. Several investigators[44-47] have observed a few human infections which apparently were acquired in these areas. The author[34] and other veterinarians have noted that some feedlot cattle in the El Centro vicinity apparently were infected. In any case, the entire Mohave and Sonoran Deserts of California are likely to be only mildly or spottily infective. This assumption is based on the indicator flora. These deserts receive the least amount of rainfall of the entire Lower Sonoran Life Zone of the United States. Rain averages only about three inches per year, and sometimes this may all fall at one time. Some years pass with no precipitation. As a result, vegetation is very sparse and soil fungi are relatively rare. (In this desert area, especially, termites serve as nature's main method of destroying dead vegetation.) Due to the virtual absence of leaching, the

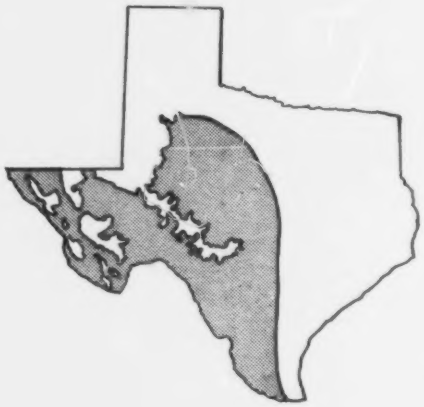

**Figure 9. Lower Sonoran Life Zone of Texas.**

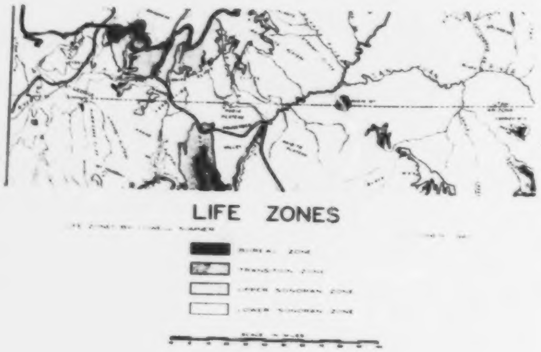

**Figure 10. Life zones of the northern Arizona and southern Utah area. The Lower Sonoran Life Zone occurs in Utah in this area only.** ***(Reproduction permission granted by the U. S. Department of Interior.)***

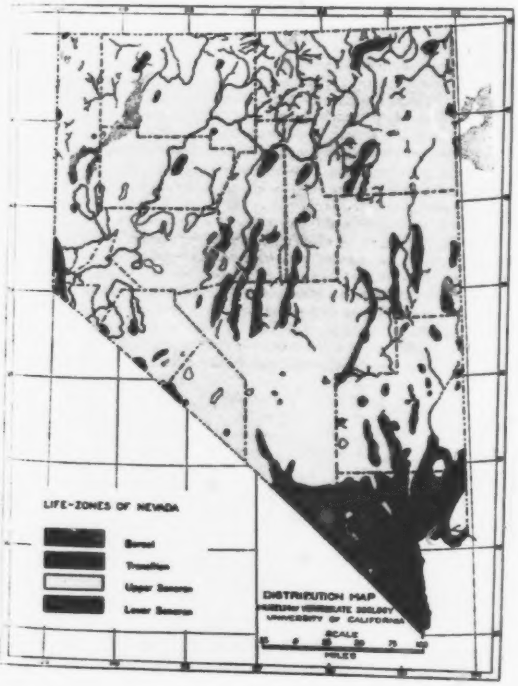

Figure 11. Life zones of Nevada. (The Lower Sonoran Life Zone is the dark area in the southern end of the State which is largely south of the 37th parallel.) *(Reproduction permission granted by University of California.)*

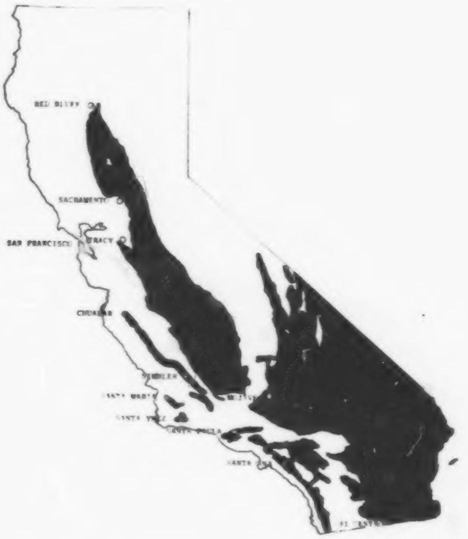

Figure 12. Lower Sonoran Life Zone in California (Black Area).

alkalinity of the area's soil remains almost as high as it was in past geologic ages when much of it was under the sea. High alkalinity is unfavorable for the growth of plants and fungi. This entire area has the almost frost-free and arid climate that apparently is so favorable for *C. immitis.* Possibly rainfall is too scarce and the soil too alkaline for abundant fungus growth as compared to the apparently more favorable conditions that exist in the nearby areas of south central Arizona and the southern end of the San Joaquin Valley.

Coastal areas in southern California, known to be endemic for *C. immitis,* correlate very closely with the Lower Sonoran Life Zone. The main infective area of the San Joaquin Valley fits almost exactly into the pattern of the Lower Sonoran Life Zone. Here (as in Texas at the east end of the Life Zone) the creosote bush does not extend all the way to the edge of the Zone. Other indicator plants and animals are found there.(20) It is interesting to see how both the Lower Sonoran Life Zone and coccidioidomycosis endemic areas extend to a point just north of the highway between Tracy and Livermore. Infective areas and the Zone correlate very well in the coastal valleys. The northern end of the Life Zone begins just south of Salinas and extends down the valleys, including the towns of Paso Robles, Shandon, and Simmler. Neither the known infective area nor the Lower Sonoran Life Zone extends west to San Luis Obispo or Santa Barbara.

The Lower Sonoran Life Zone, as designated by Grinnell,(43) extends up the Sacramento Valley to Red Bluff. Smith(48) has noted several isolated human cases of coccidioidomycosis that apparently originated in Yolo County of this area. The author and other veterinarians have noted the infection in a few feedlot cattle which evidently were infected in the Sacramento Valley. Although this area was designated as within the Lower Sonoran Life Zone and its soil may possibly support the growth of *C. immitis,* it is not nearly as suitable for the fungus as south central Arizona or the southern part of the San Joaquin Valley. Throughout both the Sacramento and San Joaquin Valleys, the high temperature is relatively similar to that in the "ideal" area in Arizona. The heaviest infectivity in California is in the southern part of the San Joaquin Valley, with the susceptibles becoming infected at the rate of about 25 percent per year.(29) As rainfall increases in the northern part of the San Joaquin Valley, the infection rates for coccidioidomycosis decrease. In the Sacramento

Valley the areas within the Lower Sonoran Life Zone receive more rainfall than the San Joaquin Valley to the south. It may be that the increase in the rainfall farther north permits another and more competitive flora to crowd out *C. immitis*.

**Discussion**

In ages past the present southwestern desert areas of the United States had a humid climate. With the change to an arid and semiarid area, only those organisms that could adapt to the new climate were able to survive. Aridity is said to serve as a stimulus to plant evolution.(49) Thus eventually it is likely that specially adapted animals and plants evolved.

The infective area of coccidioidomycosis extends down into the desert areas of Mexico.(50) In addition there are infective areas in Paraguay,(51) Argentina,(52) and Venezuela.(53) One of the main indicator plants, the creosote bush,* also exists in the desert areas of South America.

The work of Emmons(54) indicates that possibly some of the desert-living rodents have severe disseminated infections. Many species of these small mammals live entirely within the Lower Sonoran Life Zone.

Very hot summer months, mild winter weather, a period of precipitation, a soil with sparse vegetation which results in much dust, and winds are favorable for a *C. immitis* endemic area.(55)

In a soil-sampling survey for *C. immitis* in an area of the southern San Joaquin Valley of California,(56) more soil specimens were found positive at the end of the rainy season than at the end of the dry season. Soil specimens taken in or near rodent holes, especially at the end of the wet season, were more likely to be positive than samples taken from soil sites at random.

*C. immitis* grows better in sterilized soil than in nonsterilized soil, and it grows on sand as well as on dead vegetation.(57) It has been demonstrated in the laboratory that soils from various places in the United States support the growth of *C. immitis* and that temperatures as high as 42° C. and as low as -20° C. do not destroy the fungus as long as it is already growing in a soil sample. The fungus grows over all the pH ranges found in most soils.

Although the ideal conditions for maximum growth and sporulation of *C. immitis* can be determined in the laboratory, the true ecology of this fungus can be learned only by studying the organism in its natural habitat.(47)

In a study of the effects of various temperatures, humidities, and amounts of salt in the media on one strain of *C. immitis*,(58) dry spores were stored at temperatures of from -15° C. to 37° C. with relative humidities from 10 to 95 percent. There was little loss in viability at any of the temperatures less than 37° C. until the sixth month at which time there was a decrease of not more than 50 percent of the initial count. Ten percent humidity and 37° C. were deleterious but all the spores were not killed until 6 months had passed. At 50° C. all the spores were dead in 2 weeks. Long storage of dry spores followed by subjection to extremes of temperature resulted in little loss of viability unless followed by a second storage period. Storage of spores in physiologic saline solution for similar temperatures and time periods resulted in more rapid loss of viability under extremes of temperature; at 4° C. the spores were just as viable 6 months later. Spores suspended in distilled water at 4° C. remained viable for 11 months. In saturated sodium chloride solution at 4° C. there was no loss of viability in 6 months; at room temperature there was little loss of viability; but at 37° C. the rate of death was more rapid. These studies indicate that *C. immitis* has adaptations that help it to survive in its arid habitat.

Although it is still too early to summarize the data in detail, studies and observations indicate a number of ecological factors regarding the growth and propagation of *C. immitis*. In addition to man, many mammals in the endemic areas become infected. So far it appears that *C. immitis* can be isolated with some certainty only from soils in and around rodent burrows in the desert.

Apparently *C. immitis* can grow only where there is a definite period of very hot weather when there is little or no rainfall. During this time the surface soil becomes somewhat sterilized. *C. immitis* seemingly remains viable just below the sterile layer of soil, as well as in the moister and more nitrogen-rich environment of desert rodent holes. When rain eventually falls, the humidity in the surface soil probably approaches the optimum for the growth of the fungus. *C. immitis* then grows well until other soil microorganisms interfere with its growth or until the soil dries. The fungus probably still grows for a while down in cracks and holes until the humidity in these sites drops and/or other soil microorganisms interfere with its growth. Then the environment is the most infective with winds picking up dust and arthrospores and carrying them about. A study of prevailing winds over the entire endemic area does not give any over-all clue to the spread of this fungus.(6) Possibly the fungus cannot remain viable for long periods of time when airborne. A majority of human and animal infections seem to occur during the windy, dusty weather following the wet season. The sun again begins sterilizing the surface soil and the infection rates begin to drop. Infections can still occur in the seasons between peaks of infectivity. These are sometimes associated with soil digging and the consequent exposure to viable spores. Infections are often seen in dogs that dig in rodent holes while hunting on the desert, or that are taken into new home subdivisions where the soil has been graded. People too sometimes become infected in these new developments. An outbreak in a large group of airmen who dug a ditch across an airbase near Tucson, Arizona,(59) and an outbreak in students who excavated a building foundation near Bylas, Arizona,(60) are examples, as well as others such as that of a group of students digging out a snake.(61)

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*The South American plant is considered to be a different species by some botanists.

A study of various subclimates within the Lower Sonoran Life Zone is of interest. As an example, the following three areas may be compared climatically: 1. St. George, Utah, which is just within the northern limit of the Lower Sonoran Life Zone in Utah and which is mildly infective for coccidioidomycosis and is also about the northern limit for the endemic area in that part of the country; 2. Florence, Arizona, which is apparently the most infective area presently known for coccidioidomycosis; and 3. Yuma, Arizona, a hot and quite arid area which is apparently only mildly infective for coccidioidomycosis (table 1). Study of data of this type will no doubt elucidate the factors in nature that affect the growth of *C. immitis*. It appears that the coldest area that will support this fungus has a mean minimum January temperature of 25° F. Arid and semiarid areas with warmer temperatures probably can support growth of this fungus. Although the temperatures at Yuma and Florence are quite similar, there is considerably more rainfall at Florence. Gila Bend, about halfway between these places, has a mean annual rainfall of 5½ inches. Gila Bend's temperatures are very similar to those of Florence and Yuma. The Gila Bend area is somewhat infective for coccidioidomycosis. Perhaps about 5 inches of rainfall per year in these high temperature areas are just enough to create an endemic area. It appears that the best conditions for the growth of *C. immitis* are produced in the hot-weather areas that have 5 to 20 inches of rainfall.

Apparently the exact rainfall is not as important as the precipitation effectiveness which is determined by run-off, evaporation, temperature, vapor pressure, and other factors such as the season of rainfall. Suitable moistening of the topsoil at the time of the year or season when the temperatures are most ideal results in the development of humidities in topsoil that are optimal for certain plants, fungi, and bacteria.(68)

The exact measured rainfall in the three principal endemic areas in the United States is different, but the effective precipitation appears to be much less different.(67, 24) The soil type in an area has a good deal to do with water-holding capacity. The soil group that is almost synonomous with the Lower Sonoran Life Zone is designated as the "reddish soils of the semiarid to arid Southwest". This group is made up of red desert, reddish-brown, and non-calcic brown soils with much lithosol.(68) In local areas the soil can vary considerably in type and consistency and consequently in the amount of rainfall necessary to maintain a given amount of moisture in the topsoil for a given period of time.

The three heavily endemic areas for coccidioidomycosis in the southwest as indicated by Edwards and Palmer's(69) skin-test study include Kern County in California, Pima, Pinal, and Maricopa Counties in Arizona, and the counties in Texas starting at the southeast corner of New Mexico and running southeast to below Laredo. A study of data from representative weather stations in these areas is of interest (table 2).

More than about 25 inches of rainfall per year in a hot climate appears to be unfavorable for propagation of this fungus.

No doubt further studies will shed more light on those ecological factors which determine the growth, propagation, and dissemination of *C. immitis* in nature.

## Summary

A close correlation has been observed between the geographic areas of the Lower Sonoran Life Zone and the known endemic areas for coccidioidomycosis in the United States. There are indi-

## TABLE 1

## SELECTED CLIMATIC DATA* FOR ST. GEORGE, UTAH, FLORENCE, ARIZONA, AND YUMA, ARIZONA, FOR THE PERIOD 1931 - 1952 (ALL TEMPERATURES ARE GIVEN IN FAHRENHEIT)

| Climatic Factors | St. George, Utah | Florence, Ariz. | Yuma, Ariz. |
|---|---|---|---|
| Mean maximum temperature for July | 101.4 | 106.6 | 105.5 |
| Mean temperature for July | 83.4 | 90.3 | 89.6 |
| Mean maximum temperature-annual | 88.4 | 87.3 | 87.0 |
| Mean temperature-annual | 60.8 | 69.8 | 69.8 |
| Mean maximum temperature for January | 52.5 | 66.0 | 67.1 |
| Mean minimum temperature-annual | 44.1 | 52.3 | 52.5 |
| Mean temperature for January | 38.9 | 50.7 | 51.0 |
| Mean minimum temperature for January | 25.3 | 35.4 | 35.0 |
| Mean relative humidity-5:30 a.m. July | 61%** | 59%* | 57% |
| Mean relative humidity-5:30 p.m. July | 44%** | 30%*** | 32% |
| Mean relative humidity-5:30 a.m. January | 82%** | 83%*** | 71% |
| Mean relative humidity-5:30 p.m. January | 68%** | 52%*** | 48% |
| Annual rainfall (inches) | 8.84 | 9.73 | 3.32 |
| Mean number of days above 32° F. annual | 210 | 345 | 362 |
| Altitude in feet above mean sea level | 2880 | 1498 | 199 |

* U. S. Department of Commerce, Weather Bureau Stations.(62-66)

** Humidity data is for Milford, Utah, the nearest weather station to St. George with detailed humidity studies. (This data is included with some reluctance because the altitude is 5028 and the mean temperatures are about 10 degrees lower at Milford than at St. George.)

*** Humidity data is for Phoenix, Arizona, the nearest weather station with detailed humidity studies.

## TABLE 2

## CLIMATE SUMMARY FOR WEATHER STATIONS AT BAKERSFIELD, CALIFORNIA FLORENCE, ARIZONA, AND DEL RIO, TEXAS(70)

| County and State | Station | Temperature | | | | | Killing Frost Average Dates | | | | Average Precipitation | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Length of Record | January Average | July Average | Maximum | Minimum | Length of Record | Last in Spring | Last in Fall | Growing Season | Length of Record | January | February | March | April | May | June | July | August | September | October | November | December | Annual |
| | | Yr. | °F. | °F. | °F. | °F. | Yr. | | | Days | Yr. | In. | In. | In. | In. | In. | In. | In. | In. | In. | In. | In. | In. | In. |
| Kern California | Bakersfield | 38 | 47.0 | 83.5 | 118 | 13 | 36 | Feb. 21 | Nov. 25 | 277 | 40 | 1.06 | 1.08 | 1.06 | .59 | .42 | .07 | .01 | .01 | .11 | .33 | .54 | .84 | 6.12 |
| Pinal Arizona | Florence | 22 | 50.6 | 90.0 | 117 | 11 | 28 | Mar. 12 | Nov. 17 | 250 | 25 | 1.09 | .91 | 1.00 | .40 | .18 | .16 | 1.43 | 1.58 | .87 | .36 | .85 | 1.48 | 10.31 |
| Val Verde Texas | Del Rio | 33 | 52.3 | 86.3 | 111 | 12 | 33 | Feb. 22 | Nov. 29 | 280 | 33 | .54 | .71 | .89 | 1.48 | 2.42 | 2.29 | 2.17 | 1.35 | 2.47 | 2.18 | 1.17 | .82 | 18.49 |

cations that only within this Life Zone can *C. immitis* propagate in nature. A preliminary study reveals this probably reflects the need of the fungus for arid or semiarid climate, alkaline soil, relative freedom from severe frosts, and a very hot, dry season of several months followed by some rain. July mean temperatures from 26° C. to about 32° C., January mean temperatures from 4° C. to about 12° C., and an annual rainfall of about 5 to 20 inches seem to be the most ideal for the propagation of the fungus in nature. Those portions of the Lower Sonoran Life Zone with more arid or less arid climate or with lower summer mean temperatures are apparently less favorable for the fungus.

New areas of the Lower Sonoran Life Zone that had not been delineated as endemic zones for coccidioidomycosis have been discovered. Other areas where *C. immitis* infections could be expected to occur have also been designated.

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# Ecological Studies of *Coccidioides immitis*

Orda A. Plunkett, Ph.D., and F. E. Swatek, Ph.D.

In May 1954, an anthropology class from the University of California at Los Angeles excavated an old Indian camp site near Inyokern, California. Two weeks later four members of the class and a fifth student, who had later cleaned the artifacts collected, developed primary pulmonary coccidioidomycosis. Soil samples taken from the excavated area were positive for *Coccidioides immitis*. During the summer and fall of 1954, rodents were trapped and soil samples were taken from an area ½ mile wide and 5 miles long, extending ½ mile south and 4½ miles north of the camp site. Eighty composite soil samples taken from walls of rodent burrows in this area away from the immediate vicinity of the camp site were negative. Four hundred forty kangaroo rats (*Dipodomys* sp.) and pocket mice (*Perognathus* sp.), as well as numerous other rodents, were trapped, autopsied, and their lungs examined and cultured for *C. immitis*. None of the rodents was positive for *C. immitis*. From these preliminary studies it was decided that the endemic area was limited to a single pocket, and on-the-spot check ecological studies of this area were made.

The camp site fronts on a shallow cave situated on a sandstone ridge in the El Paso mountain range at an elevation of about 3200 feet, 10 miles south of Inyokern, California (fig. 1). The cave front opens to the southeast and is flooded with sunlight many hours of the day. The soil of the immediate camp site is composed of a mixture of disintegrated sandstone, campfire debris, and decayed and partially decayed plant remains. The soil could be classified as a sandy loam and when dry is freely blown by the wind. The soil has good water-holding capacity, and moisture is evident 8 to 10 inches below the surface throughout the year and can usually be found at the 4- to 6-inch level except in August, September, and October. The average annual rainfall here of 4 to 8 inches tends to occur in the form of sudden cloudbursts during the summer months and in gentle rains during the winter months. The wind blows continually across the camp site. Soil temperatures 1 inch below the surface range from below freezing to 60.5° C. during the year. Soil temperatures at different sampling depths for March, April, and May are given in table 1.

Vegetation of the camp site is scant, and rodent burrows are few and apparently abandoned as no animals were trapped here. Insects are not abundant and are represented by ants, ground beetles, and deer flies. Birds have been observed to dust themselves in the cave frontage.

## Materials and Methods

Soil samples were taken in sterile, 4-ounce, screw-capped jars by making a profile with a shovel and forcing the jar into the soil at the surface and at 4-inch and 6-inch levels. Rocks and large pieces of plant debris were removed at the time of sampling.

In the laboratory soil samples were well mixed, and 10 grams of the soil sample were added to 90 cc. of sterile, distilled water to make a 1 to 10 dilution. In the early studies 1 to 100 and 1 to 500 dilutions were used but were later abandoned as being unsatisfactory. The most satisfactory results were obtained by using the 1 to 10 dilution in water heated to 40° C. The dilutions were shaken up and allowed to stand for 30 minutes before being sprayed on the surface of hardened agar plates. Eight milliters of Sabouraud's glucose agar fortified with penicillin, streptomycin, and cycloheximide* (P.S.C.) were poured into 60 mm. Petri dishes and allowed to harden. Five plates were made of each soil dilution. The soil dilutions were sprayed onto the hardened agar surface.

The inoculated plates were incubated at room temperature from 10 to 21 days and frequently examined for colonies of *C. immitis*.

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*From the Department of Botany, University of California, Los Angeles, California.*

*Trade name, Acti-dione, Upjohn Co., Kalamazoo, Mich. Trade names are used for identification only and their use does not constitute endorsement of the product by the Public Health Service.

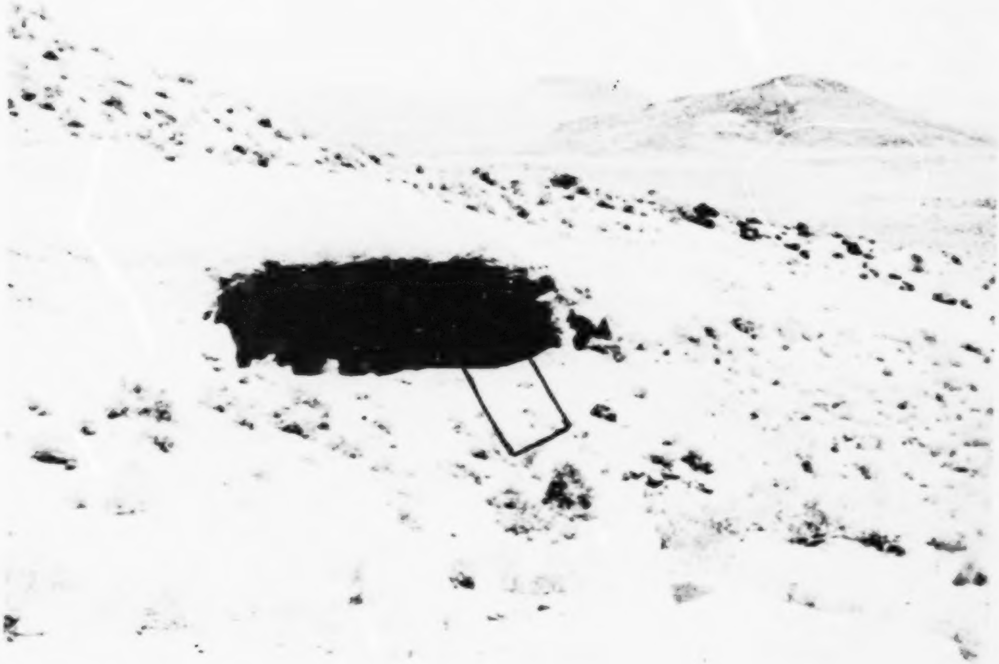

Figure 1. Cave at Indian camp site near Inyokern, California, showing nature of terrain and scant vegetation. The inked-in rectangle shows extent of infected soil.

## TABLE 1

## SEASONAL SOIL TEMPERATURES AT VARIOUS DEPTHS

| Month | Soil Depth | | | |
|---|---|---|---|---|
| | 0-2 Inches | 4-6 Inches | 8-10 Inches | 18 Inches |
| March | 40° C. | 12° C. | 10° C. | 10° C. |
| April | 40° C. | 25° C. | 17° C. | 12° C. |
| May | 42° C. | 31° C. | 26° C. | 15° C. |

## Soil Sampling Results

Miscellaneous soil sampling of the area, done over a period of a year, had shown that positive soil samples could only be obtained from a limited area of about 6 feet by 10 feet directly in front of the cave. Soil samples were taken each month from 3 stations approximately 3 feet apart in a line through the center of the infected area. Three samples were taken from each station at the surface, at 4-inch, and at 6-inch depths (table 1). A composite sample from four outlying areas was included each month.

Our method of soil sampling may not have produced as accurate results as could have been obtained by using the mouse technique; however, repeated culturing of several samples has given comparable results. Dilutions of known positive samples were run each month as checks. See table 2.

## Summary

Apparently *C. immitis* is able to maintain itself when it is established to a depth of 6 to 8 inches in soils, although viable spores are absent from the surface layer during the hot summer months. A high temperature of 60.5° C. was recorded on a thermometer buried 1 inch deep in the soil in front of the cave, and temperatures of 49° C. to 54.4° C. were frequently registered.

Although the fungus is known to be airborne and high winds are frequent in the area studied, *C. immitis* infected soil was found only in small pockets. Repeated sampling of soils from the immediate vicinity and surrounding territory have never produced a positive sample. It appears that there is some unknown factor in the environment which favors the growth of this fungus in certain definite habitats.

TABLE 2

SEASONAL RECOVERY OF *COCCIDIODES IMMITIS* FROM SOIL SAMPLES

| Depth | Month | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | June | July | Aug. | Oct. | Nov. | Dec. | Jan. | Mar. | Apr. | May |
| Surface | + | + | – | – | – | + | + | + | + | + |
| 4 Inches | + | + | + | + | + | + | + | + | + | + |
| 6 Inches | – | – | + | + | + | + | – | + | + | + |

# Ecological Studies on *Coccidioides immitis*

## *Experimental Infections of Wild Rodents and Animals Other Than Mammals*

F. E. Swatek, Ph.D., and Orda A. Plunkett, Ph.D.

The present studies were designed to investigate the pathogenicity of *Coccidioides immitis* for the wild rodents of southern California and to investigate the survival of the fungus in animals other than mammals.

Since 1952 this laboratory has conducted soil sampling and rodent-trapping surveys in endemic areas. The results show that only a small percentage of the rodent species are naturally infected. Among these are the kangaroo rat (*Dipodomys* sp.), pocket mouse (*Perognathus* sp.), and jack rabbit. The susceptibility of rodents to *C. immitis* infection was determined under laboratory conditions. Wild rodents captured in traps were brought to the laboratory and acclimated for several weeks to be sure that none of them were naturally infected. The rodents were then inoculated intraperitoneally with 0.25 ml. of an arthrospore inoculum. All of the rodents so inoculated (table 1) succumbed to the infection within periods of from 5 days (mice) to 94 days (a long-tailed ground squirrel).

From the evidence at hand, the animals infected in nature have primary pulmonary infections that do not disseminate but remain in the lungs and probably heal. In man it has been found that such primary pulmonary infections are usually self-limiting and seldom become disseminated to produce a systemic infection. None of the wild rodents collected and retained in the laboratory died of coccidioidomycosis. However, all animals injected intraperitoneally with *C. immitis* succumbed with a disseminated infection, the death rate being governed by the size of the inoculum. In nature the two most susceptible species are the pocket mouse and the kangaroo rat. Both of these rodents habitually throw large quantities of dirt with their hind legs while digging their burrows as a defense against enemies, and this active disturbance of the soil may explain their higher percentage of infections over such neighbors as the wood rats and white-footed mice. Digging could also explain the occurrence of infections in some of the other rodents such as rabbits and squirrels whose diet includes roots. Rodent infections in nature would seem to be incidental, with no more harmful consequence than a weakening of the animals. If the disease is self-limiting, as the evidence suggests, the role of the rodent in the ecology of this disease is that of victim and is insignificant as a reservoir in nature.

### Experimental Infections in Crayfish

As a result of the experimental inoculations on rodents, it was decided to try similar experiments on animals other than mammals. Two lots of mature crayfish (*Cambarus* sp.) were collected and placed in an aquarium for two weeks before being inoculated. These invertebrates were held ventral side up and 5 ml. of 70 percent alcohol were allowed to run freely over their surface and allowed to evaporate. This accomplished two things: It partially sterilized the crayfish and partially anesthetized them. The inoculum was deposited in the coelomic cavity in the anterior thoracic region.

The results of the inoculation of 12 crayfish in the first series are given in table 2.

Cultures of material from the digestive organ, muscle, gonads, and gills were positive. Sections were made from material of the crayfish killed after 42, 67, and 110 days. Nodules were found embedded in the fascial tissue around the outside of the abdominal material, and in the gill tissue and gonads (fig. 1). These nodules contained many large arthrospores. The largest spherical spores were examined, but none was found with cleavage planes (fig. 2). A dark melanin pigment deposit was found associated with these nodules. Many arthrospores were found inside white cells.

In series II, crayfish were inoculated in a manner similar to that of series I, using 0.5 ml. of

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*From the Department of Botany, University of California, Los Angeles, California.*

## TABLE 1

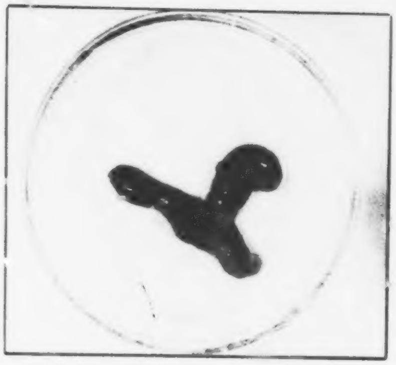
## WILD RODENTS SUSCEPTIBLE TO *COCCIDIOIDES IMMITIS* UNDER LABORATORY CONDITIONS

| Scientific Name | Common Name |
|---|---|
| *Citellus* sp. | Ground squirrel |
| *Ammospermophilus* sp. | Antelope chipmunk |
| *Dipodomys* sp. | Kangaroo rat |
| *Perognathus californica* | Allen pocket mouse |
| *Perognathus* sp. | Tejon pocket mouse |
| *Reithrodontomys* sp. | California harvest mouse |
| *Peromyscus* sp. | Southern parasitic mouse |
| *Peromyscus eremicus fraterabus* | Dulzura white-footed mouse |
| *Peromyscus maniculatus gambeli* | Gambel white-footed mouse |
| *Neotoma lepida* | Desert wood rat |
| *Neotoma fuscyses macrotis* | San Diego wood rat |
| *Microtus californicus sanctidiego* | Southern California meadow mouse |

## TABLE 2

## RESULTS OF INOCULATION OF CRAYFISH WITH *COCCIDIOIDES IMMITIS*

| No. of Crayfish | No. of Days After Inoculation | Culture Results | KOH Findings |
|---|---|---|---|
| 4 (died) | 1 - 5 | + | No apparent pathology |
| 1 | 9 | + | No apparent pathology |
| 1 | 19 | + | No apparent pathology |
| 1 | 27 | + | Arthrospore-filled nodules noted in gills and abdominal organs |
| | | + | |
| 1 | 35 | + | |
| | | + | |
| | 42 | | |
| 1 | 67 | + | Abdomen and gills well spotted with nodules |
| 1 | 110 | + | Nodules pronounced and very abundant |



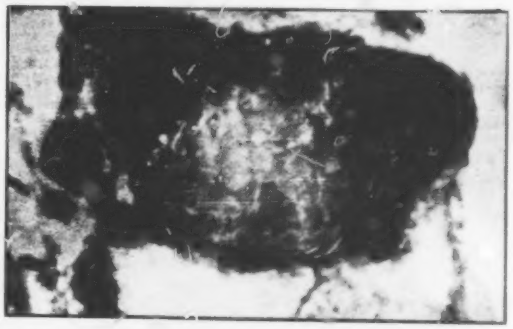


Figure 1 (left). Dark nodules on infected gonad from crayfish. Figure 2 (right). Nodule embedded in fascial tissue of crayfish containing rounded and enlarged arthrospores.

inoculum. Special care was used to remove the carapace to expose the gill. Gill material was then removed for plating, sectioning, and KOH observations. After the gill material was removed, the abdomen was opened and examined for lesions. Dark colored nodules containing enlarged arthrospores and filaments were found scattered among the viscera (fig. 3). All of the crayfish gave positive culture results. KOH mounts revealed arthrospore clusters in all crayfish examined in the second series.

Sections were made of various tissues and organs. Stained arthrospores and mycelial filaments were seen in varying stages of enlargement (fig. 4). Cleavage planes were never observed in the enlarged spores. Evidence of mycelial growth was present in one crayfish in which a necrotic lesion was observed. This lesion connected the abdominal cavity with the gill chamber and one gill filament was found firmly adhering to the necrotic area (fig. 5).

A gill filament was removed aseptically from a crayfish 42 days after inoculation, placed in sterile saline, and macerated. This suspension was injected into two mice using 0.3 ml. aliquots. One mouse was sacrificed seven days later. Autopsy revealed a multitude of small whitish-yellow lesions on the liver, omentum, diaphragm, and spleen. KOH preparations of this material contained spherules in all stages of development, with a predominance of the central vacuole type.

The second mouse died on the tenth day after inoculation, and it had extensive lesions. These lesions appeared similar to those in the first mouse, and the presence of spherules was confirmed by KOH mounts. Material from these two mice yielded positive cultures.

### Experimental Infections in Goldfish (*Carassius* sp.)

A series of 8 goldfish, 4 to 6 inches long, were inoculated with 0.5 ml. of inoculum from a crayfish isolate of *C. immitis* in the same manner. These fish were examined for gross pathological findings. KOH mounts were made, and some material was sectioned for study of the progress of the disease.

Each fish was examined for pathological findings by making incisions along the mid-ventral line and at right angles to the backbone at the anus and gill operculum. The flap was then lifted back and examined. Lesions occurred in the form of scattered dark nodules as shown in figure 6. When crushed, these nodules revealed single arthrospores, short filaments, and aggregates of arthrospores. Some of the mycelial fragments were enlarged and rounded up. None of the small, rounded cells were found to contain cleavage planes. In sectioned material some of the nodules were found walled off and others were rather loosely constructed. Arthrospores were found inside white cells, yet their staining characteristics showed them to be viable.

In some areas the fungus was found free among, but not engulfed by, white cells (fig. 7). Most of the nodules were found in the fascial membranes surrounding the internal organs (fig. 8). In the older fish lesions were found in which the fungal elements were being absorbed (fig. 9).

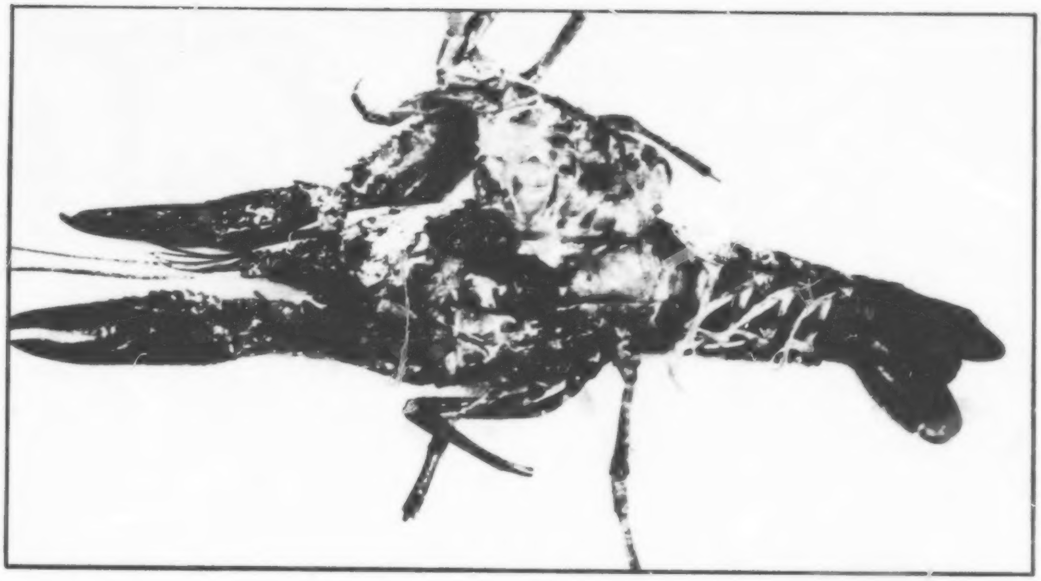


Figure 3. Dissected crayfish showing lesions in viscera caused by *C. immitis*.

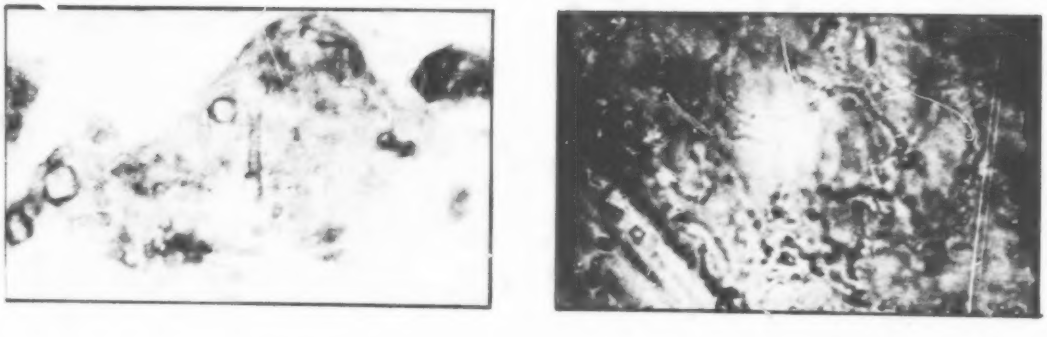


Figure 4 (left). Nodule containing enlarged mycelial fragments and arthrospores in gill tissue of crayfish. Figure 5 (right). KOH mounts of necrotic lesion from a crayfish showing branching mycelium and enlarged arthrospores.

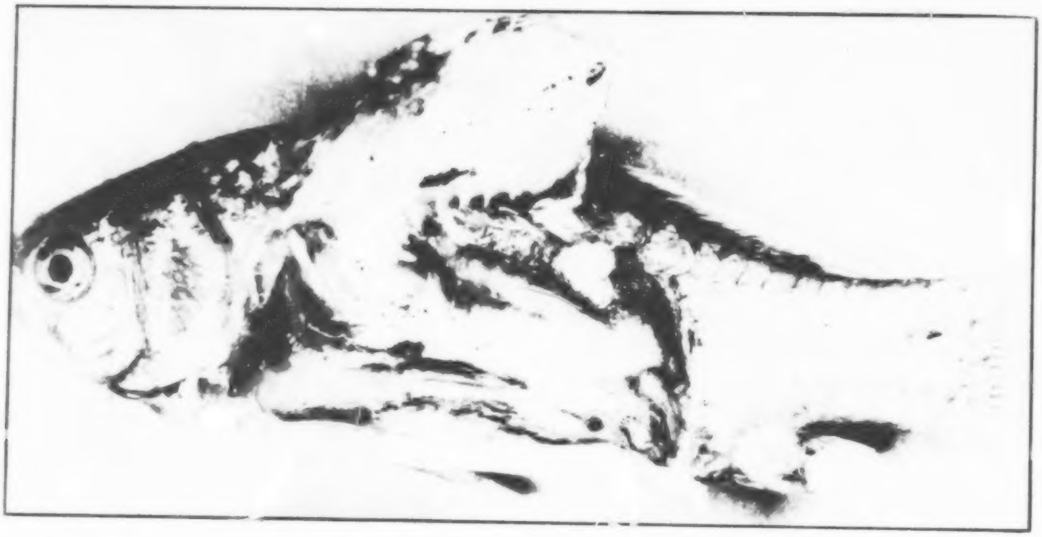
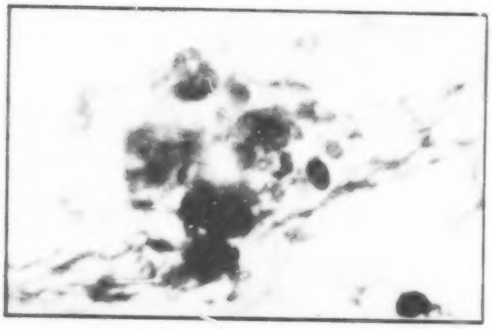
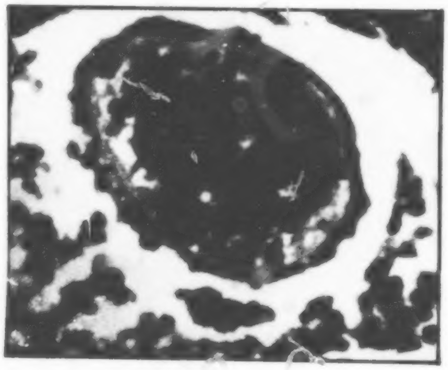


Figure 6. Dissected goldfish showing dark nodules in the abdominal cavity.





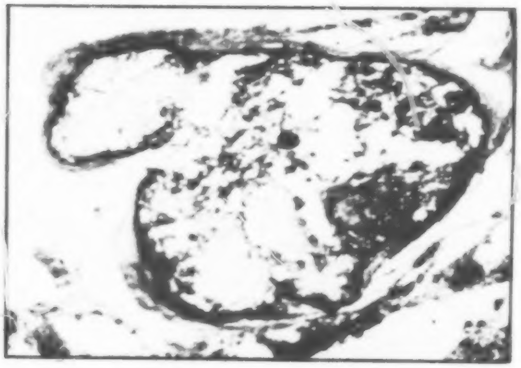


Figure 7 (upper left). Aggregation of arthrospores surrounded by white cells in goldfish tissue. Figure 8 (upper right). Nodule embedded in fascial membrane of goldfish containing rounded, viable arthrospores. Figure 9 (lower left). Large nodule from goldfish containing amorphous material from disintegrated arthrospores.

## Experimental Infections in Lizards

Thirteen small western fence lizards (*Sceloporus* sp.), two large alligator lizards (*Sceloporus* sp.), and a horned toad (*Phrynosoma* sp.) were inoculated intraperitoneally with *C. immitis*. Table 3 shows the death rate of the western fence lizards. Five days after inoculation one lizard died. KOH mounts of the lung tissue revealed well developed spherules in early stages of cleavage (fig. 10). The material was plated on Sabouraud's glucose medium and positive cultures were recovered. Two lizards that died six days after inoculation were found to contain many enlarged spherules with mature endospores. Some sporangia were ruptured, others were still attached to their connective cells in the mycelium.

An alligator lizard and the horned toad were killed 17 days after inoculation. The lungs, liver, and fascial tissue of the viscera were found to contain spherules. In these tissues the spherules were mostly in the early cleavage stage; however, mature ones containing endospores were present. The disease appeared to progress at a slower rate in these reptiles than in the smaller western fence lizards.

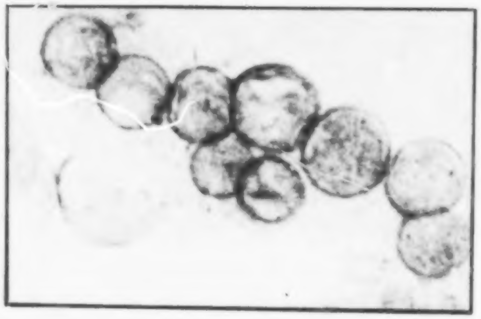


Figure 10. KOH mount of lung tissue from a lizard showing spherules in various stages of development.

Lizards have well developed liver and lung tissue. *C. immitis* produces a pathology in these animals identical to that which occurs in mammals. The fungus develops very rapidly and produces a chronic, disseminated infection. Neural distrubances were observed in two lizards before death.

### TABLE 3

### DEVELOPMENT OF *COCCIDIOIDES IMMITIS* INFECTIONS IN LIZARDS

| No. of Days After Inoculation | No. of Animals That Died | Culture Results | KOH Observations |
|---|---|---|---|
| 5 | 1 | + | Young spherules present in lung tissue |
| 6 | 2 | +<br>+ | Spherules found in all all stages of development |
| 7 | 1 | + | |
| 8 | 1 | + | All stages of spherules present in lungs and liver |
| 10 | 1 | + | |
| 11 | 1 | + | Spherules in all stages present in lungs, liver, and fascial membranes of viscera. Second generation of spherules present in some lizards |
| 12 | 1 | + | |
| 13 | 1 | + | |
| 14 | 1 | +<br>+<br>+ | |
| 17 | 3 (killed) | + | |

## Discussion

Studies of the growth of *C. immitis* in crayfish showed the production of the saprophytic mycelial stage, and not the spherule or normal mammalian parasitic stage. Dissemination was extensive involving the gills as well as the viscera. The fungal spores were phagocytized and transported to all areas of the body. Some spores were seen to enlarge and round up, others to elongate, still others to germinate and produce mycelium (fig. 5). The fungus remained viable even up to the death of the animal. Our observations are not extensive enough for us to say positively that *C. immitis* was responsible for their death. However, there was only a five-day interval between the death of the last animals in two independent experiments.

*C. immitis* exists in the mycelial phase in goldfish and crayfish. The infections differ, however, in the two animals since *C. immitis* was restricted to the abdominal viscera of the fish, and the gills did not become involved. Here again rounded cells and elongated mycelial filaments were found (fig. 4).

Schlumberger,(1) studying a case of meningeal coccidioidomycosis, infected white mice and frogs intraperitoneally. He observed typical spherule formation in the mice. He found small lesions containing arthrospores and some enlarged, rounded cells in the abdominal cavity of frogs. A few of these enlarged cells possibly had a single cleavage plane.

The factors necessary for spherule production are unknown. Other diphasic organisms are known to require an elevated temperature to maintain the parasitic stage. Several investigators have grown spherules *in vitro* at elevated temperatures. Lubarsky and Plunkett(2) have shown the importance of the proper $CO_2$-$O_2$ ratio for the development of spherules.

The infections in lizards were surprising since spherules were produced in these cold-blooded vertebrates at room temperatures. However, lizards are more highly evolved than fish and frogs and have better lung tissue. It is in the lung and liver tissue of lizards that the spherules thrive. One then questions the necessity of a high temperature as an essential factor in the production of spherules. Baker and Braude(3) have reported that the spherule stage is inhibited by certain white cells and stimulated by others. However, photographs of their work reveal only enlarged arthrospores and not sporangia. Some of the material in the crayfish was of a comparable nature, yet when compared to true spherules, the difference became evident.

It is interesting to assemble the animals in an evolutionary sequence and compare the development of *C. immitis* in them. The crayfish, goldfish, and frogs supported only a saprophytic-like form of *C. immitis*. Only mycelium and arthrospores were found. In lizards and mammals the sporangial form of the fungus developed. Spherules perhaps developed in the lizards since their tissue and organs are more akin to those of mammals than of amphibians. The fact that their body temperature is lower than that of mammals did not prevent the development of the parasitic phase.

## References

(1) Schlumberger, H.: A fatal case of cerebral coccidioidomycosis with cultural studies. Am. J. M. Sc. 209:483-496, 1945.

(2) Lubarsky, R., and Plunkett, O. A.: *In vitro* production of the spherule phase of *Coccidioides immitis*. J. Bact. 70:182-186, 1955.

(3) Baker, O., and Braude, A. I.: A study of stimuli leading to the production of spherules in coccidioidomycosis. J. Lab. & Clin. Med. 47:169-181, 1956.

# Growth Patterns of *Coccidioides immitis* in the Soil of an Endemic Area

Ann Frank Elconin, M.D., Roger O. Egeberg, M.D., and Robert Lubarsky, Ph.D.

In July 1955, we reported on the dramatic seasonal variation in the percentile recovery of *Coccidioides immitis* from the soil of the southwestern part of the San Joaquin Valley.[1] Of the soil samples taken in mid-January 1954 (at the end of that year's dry season), we found 4.2 percent to be positive for *C. immitis*. However, this fungus was present in 16 percent of the samples collected early in April 1955 (following the wet season). It was found that regardless of the season, the percentage of positive cultures was proportionately higher in samples taken from the lining of rodent burrows than in random surface or subsurface soil samples. In the total samplings, of which 7 percent were positive for *C. immitis*, 13.6 percent of the samples taken from the rodent burrows were positive, compared to 3.4 percent taken at random.

Because of the apparent growth habits of this fungus within the soil, it was decided to continue this study in the same manner through another seasonal cycle. Although we speak of years and dates, the material should more correctly be related to seasons. Southern California has a wet season beginning in November, December, or January and usually ending in March, April, or May. This is followed by a hot, dry season which continues into the late fall months. The soil samples in this series were collected in seasons comparable to those of the first group rather than on corresponding calendar dates. These samples were obtained from the same 12-to-14-acre area near McKittrick, California.

## Experimental Methods

The procedure for collecting soil samples, the method of soil cultures, and the criteria for the identification of *C. immitis* from the cultures were the same as those described in the earlier report.[1]

## Results

A total of 428 soil samples were studied in this group, of which 31 or 7.2 percent were positive for *C. immitis*. In August 1955, at the height of the dry season and four months after the last rain, 143 samples were collected. In December 1955, toward the end of the dry season and eight months after the last rain, 132 samples were collected. In May 1956, following the rainy season and after two weeks of warm weather, 153 samples were collected.

It should be emphasized that all of the 31 positive soil specimens came from the group of 153 collected in May 1956. Hence, 20.2 percent of the soil samples taken at the end of the wet season were positive when cultured for *C. immitis* (table 1).

The fungus was not recovered from any of the 275 samples collected in August and December of 1955, during and at the end of the hot, dry season. Of the 428 soils cultured, 115 were rodent burrow samples, and of these, 18 or 15.6 percent were positive. There were 313 random surface and subsurface soils, giving 13 or 4.1 percent positive cultures (table 2).

Table 3 shows the distribution of positive cultures with respect to time and site of collection. It is noteworthy that of the 30 surface soil specimens taken in the May 1956 group, 13 or 43.3 percent were positive. Also, of the 44 burrow samples taken at the same time, 40.9 percent were positive.

## Discussion

The year-round percentile recovery of *C. immitis* from the sampling of soil was 7.2 percent and this agrees with the 7 percent reported in the first paper. The dramatic difference between samples collected during the dry season and those collected at the end of the wet season again holds

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*From the Kaiser Foundation Hospital, Los Angeles, California, and the Los Angeles County General Hospital.*

## TABLE 1
## *COCCIDIOIDES IMMITIS* IN SOIL

| Time Collected | No. of Soils | Number Positive | Percent Positive |
|---|---|---|---|
| Collected End of Dry Season | 275 | 0 | 0 |
| Collected End of Wet Season | 153 | 31 | 20.2 |

## TABLE 2
## *COCCIOIDES IMMITIS* HABITAT

| Collection Site | No. of Soils | Number Positive | Percent Positive |
|---|---|---|---|
| From Burrows | 115 | 18 | 15.6 |
| Random Samples (Surface & Subsurface) | 313 | 13 | 4.1 |

## TABLE 3
## LOCATION AND SEASONAL OCCURRENCES OF *COCCIOIDES IMMITIS* IN SOIL

| | August & December 1955 | | | May 1956 | | |
|---|---|---|---|---|---|---|
| Source | No. of Samples | No. Pos. | Percent | No. of Samples | No. Pos. | Percent |
| Surface | 53 | 0 | 0 | 30 | 13 | 43.3 |
| 4" Depth | 52 | 0 | 0 | 26 | 0 | 0 |
| 8" Depth | 49 | 0 | 0 | 27 | 0 | 0 |
| 12" Depth | 50 | 0 | 0 | 26 | 0 | 0 |
| Burrow Samples | 71 | 0 | 0 | 44 | 18 | 40.9 |

true, and again a very high percentage of positive cultures came from surface samples as compared with those taken at lower levels. Burrow soil specimens in both studies yielded a large percentage of positive cultures at the end of the wet season. In this series, although no positive cultures were found in the dry, hot season as compared with 1.3 percent reported in the first paper, one would probably be correct in assuming that the fungus still existed in quantities too small to be detected by our limited study. As stated in the first paper, the few positive cultures that were obtained during the hot season were found in the random subsurface levels. It is possible that because of the lowered water table, the fungus migrated deeper than the 12-inch level.

Our earlier postulations would seem to be supported by the second year's study. These results, when viewed against the background of the first year, would warrant the following conclusions and speculations:

1. *C. immitis* is most easily found in the soil during the first warm weeks following a rainy season.

2. At this time of year, *C. immitis* is present primarily in the top inch of soil and in the soil lining rodent burrows.

3. *C. immitis* is found in the soil only occasionally during the hot, dry season.

4. The ease with which *C. immitis* was found in the soil in two successive years would indicate that the soil is the reservoir for this fungus and that man, along with animals, are only accidental hosts.

5. Again, we raise the questions: Could it be that this scorching summer heat is necessary to the life of *C. immitis*? Does it prepare a thin layer of relatively sterile topsoil which needs only moisture to favor invasion by the fungi living below and adjacent to it? Could heat be an ally of *C. immitis* that produces, for a brief period of time, a growth medium undisturbed by antagonists and competitors?

## References

(1) Egeberg, R. O., and Ely, A. F.: *Coccidioides immitis* in the soil of the southern San Joaquin Valley. Am. J. M. Sc. 231:151-154, 1956.

# Characteristics of Skin Reactions to Coccidioidin and Histoplasmin, With Evidence of an Unidentified Source of Sensitivity in Some Geographic Areas

Carroll E. Palmer, M.D., Phyllis Q. Edwards, M.D., and William E. Allfather*

About 10 years ago it became apparent that fungus infections that produce pulmonary lesions were creating a serious problem in tuberculosis control work in this country. It was apparent also that fungus infections were closely tied to geography. In fact, place of residence seemed to be the most important single factor affecting the risk of infection.

Since it seemed desirable to try to define the regions where fungus disease was complicating the interpretation of pulmonary X-ray findings, the Tuberculosis Program of the U. S. Public Health Service began to collect information on as near a nation wide scale as possible about histoplasmin, coccidioidin, and tuberculin sensitivity. Starting with observations on student nurses attending training schools in different metropolitan areas throughout the country, the material was supplemented later with observations on students attending colleges, universities, and professional schools, mostly in the Middle West and New England. The material was further extended in 1950-1951 by observations on large numbers of young men being recruited by the Navy. Thus, over a period of about six years it was possible to collect results for histoplasmin, coccidioidin, and tuberculin tests in more than 100,000 young people from all parts of the country.

Reports of some of the results have been published during the past year.(1-3) Today, we would like to review the most critical findings and, in particular, the method of analysis that has led us to the hypothesis that a substantial amount of the sensitivity found in certain regions, including east Texas, is not caused by infection with either *Histoplasma capsulatum* or *Coccidioides immitis*. Instead, the sensitivity seems to be a result of infection with some other organism, probably another fungus; and in testing with histoplasmin and coccidioidin we are apparently using heterologous antigens.

First, we should like to discuss briefly several points in the technical procedures. Most of the coccidioidin tests were given with a 1:100 dilution of batch C-24, prepared by Dr. Charles E. Smith of the School of Public Health, University of California, and the histoplasmin tests with a 2:1000 dilution of batch H-40, prepared by Dr. Arden Howell, Jr., of the Public Health Service.

The skin reactions were read 48 to 96 hours later by measuring and recording the transverse diameter of palpable induration. Only a few people gave the tests, and it was emphasized that their job was to measure accurately the size of the induration of the reactions, never to try to classify the reactions as positive or negative, or into categories of degrees of positivity. Although the results of the analysis indicate that the measurements were not always as precise as they could have been, we believe that the errors and variations are distributed at random throughout the entire material. In other words, we have every reason to believe that differences in results for persons from different geographic areas are valid and not the result of variations in the antigens, in the technical procedures, or some kind of observer bias.

At the time of testing, each person was asked to fill out a fairly complete form listing every city or county in which he had lived since birth. From this information we could later select for analysis only those persons who had always lived in one county or had not lived outside their home county for more than six months. Because geography is such an important factor in fungus infections, our

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*Deceased.*

material for comparing results for different geographic areas is limited to around 50,000 young white adults who could be classified as lifetime one-county residents, and who, in addition, were between 17 and 21 years of age at the time they were tested.

### Results

Our results are presented on two maps showing the geographic variations in sensitivity to coccidioidin and to histoplasmin, and on a series of graphs which bring out certain characteristics of specific reactions, negative reactions, and cross-reactions to coccidioidin and to histoplasmin. Finally, as illustrated by results for native residents of eastern Texas, it appears that a fairly large number of histoplasmin reactions and a few coccidioidin reactions in some geographic areas cannot be interpreted as resulting from either histoplasmal or coccidioidal infection.

The geographic pattern of coccidioidin sensitivity among young adults is shown in figure 1. For this map, reactions of 5 mm. or more were taken as positive and the percentages of positive reactors were plotted for rather broad levels of frequency, county-by-county, throughout the country. Appreciable frequencies of coccidioidin reactors are found only in the southwestern part of the country, with the highest frequencies in the San Joaquin valley of California, in southern Arizona, and in southwestern Texas. Beginning in the middle of Texas, and extending eastward across the central part of the country, the percentage of coccidioidin reactors drops to below 5 percent; and in the Northwest and Southeast to less than 1 percent.

For histoplasmin sensitivity, the picture is quite different (fig. 2). The percentage of positive reactors – those with reactions measuring 5 mm. or more – exceeds 80 percent in several areas in the east central part of the country. Along the Atlantic seaboard and throughout the western half of the country, very few lifetime residents react to histoplasmin. In the Southwest, however, moderately high percentages of histoplasmin reactors are found in scattered areas coinciding with the highest coccidioidin areas.

Although the high prevalence area of coccidioidin sensitivity is restricted to the southwest part of the country in the San Joaquin Valley, and along the U. S.-Mexican border and the principal high prevalence area of histoplasmin sensitivity to the east central part of the country, a low frequency of coccidioidin reactors is found in the *Histoplasma* area, and a substantial frequency of histoplasmin reactors in the *Coccidioides* area.

We know from experimental studies in the labotory, and from field studies in human beings, that cross-reactions may occur between the two antigens. Some persons who are infected with *Histoplasma* may react to coccidioidin, and some infected with *Coccidioides* may react to histoplasmin. But the frequency of cross-reactions is known to differ with the two antigens. Coccidioidin cross-reactions are much less frequent in histoplasmal-infected persons than histoplasmin cross-reactions in coccidioidal-infected persons. Moreover, it is also known that reactions to the heterologous antigen (cross-reactions) are smaller in average size than specific reactions to the homologous antigen.

It might seem reasonable to assume that most, or all, of the coccidioidin reactions in lifetime residents of the east central area, and histoplasmin reactions in lifetime residents of the Southwest, are cross-reactions; and that the endemic areas for the two fungus infections are distinct and separate. If that assumption were correct, we would then expect all, or nearly all, of the coccidioidin reactions in the east central area to occur in histoplasmin reactors; and most of the histoplasmin reactions in the Southwest to occur in coccidioidin reactors. Moreover, we would expect histoplasmin reactions to be of larger average size in the east central area, where histoplasmin is presumably the homologous antigen, and of smaller average size in the Southwest where histoplasmin is presumably a heterologous antigen. For coccidioidin the situation would of course be the reverse: we would expect larger coccidioidin reactions in the Southwest and smaller ones in the east central area.

Figure 3 shows the sizes of indurations of reactions to coccidioidin in the Southwest where, in the main, we are presumably testing with the homologous antigen; that is, most of the reactions are probably due to specific coccidioidal infection. The upper histogram gives the frequency distribution of the sizes of reactions for Navy recruits who had lived all their lives either in the southern San Joaquin Valley or in southern Arizona. The frequency of positive reactors in the group is about 50 percent. The lower histogram is for recruits from southern New Mexico and western Texas, where the combined frequency of positives is about 20 percent. In both histograms the reactions are grouped by 2 mm. intervals; and we have drawn

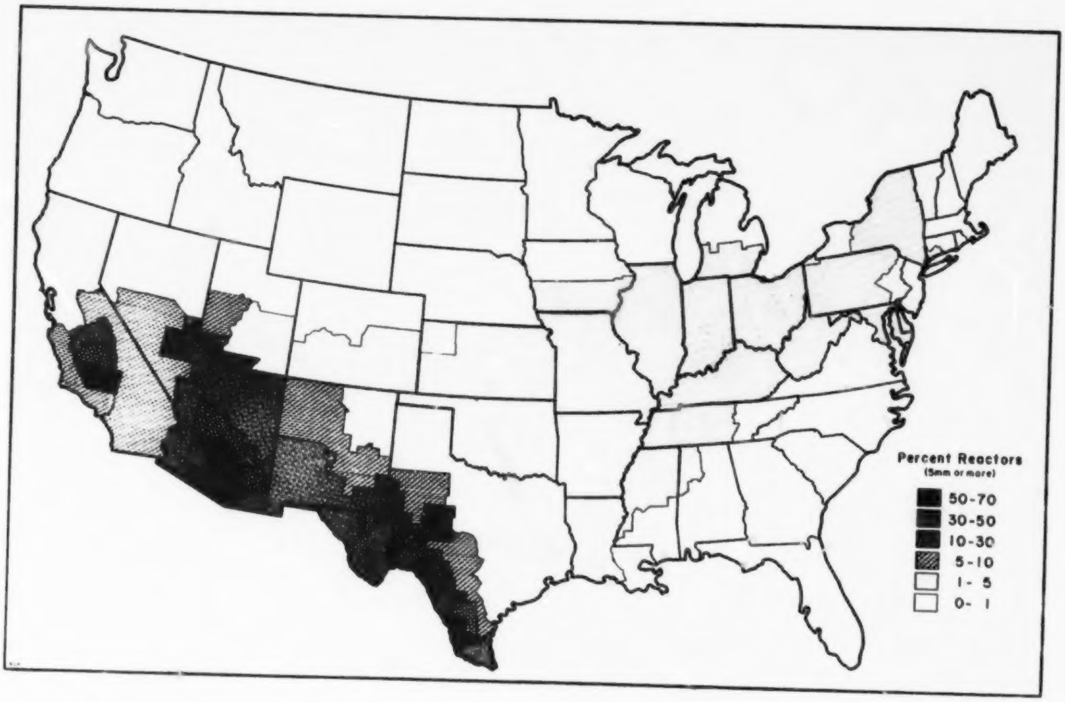

Percent Reactors
(5mm or more)
50-70
30-50
10-30
5-10
1- 5
0- 1

Figure 1. Geographic distribution of coccidioidin sensitivity in young adults. (Reproduced with permission from *Diseases of the Chest*, Volume 31, 1957.)

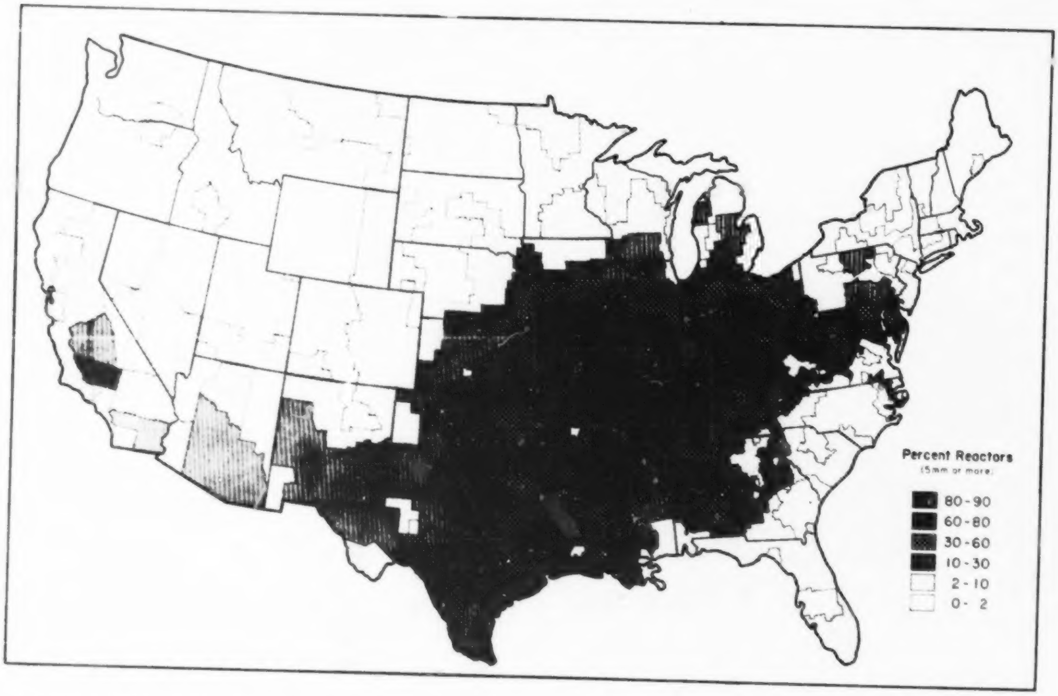
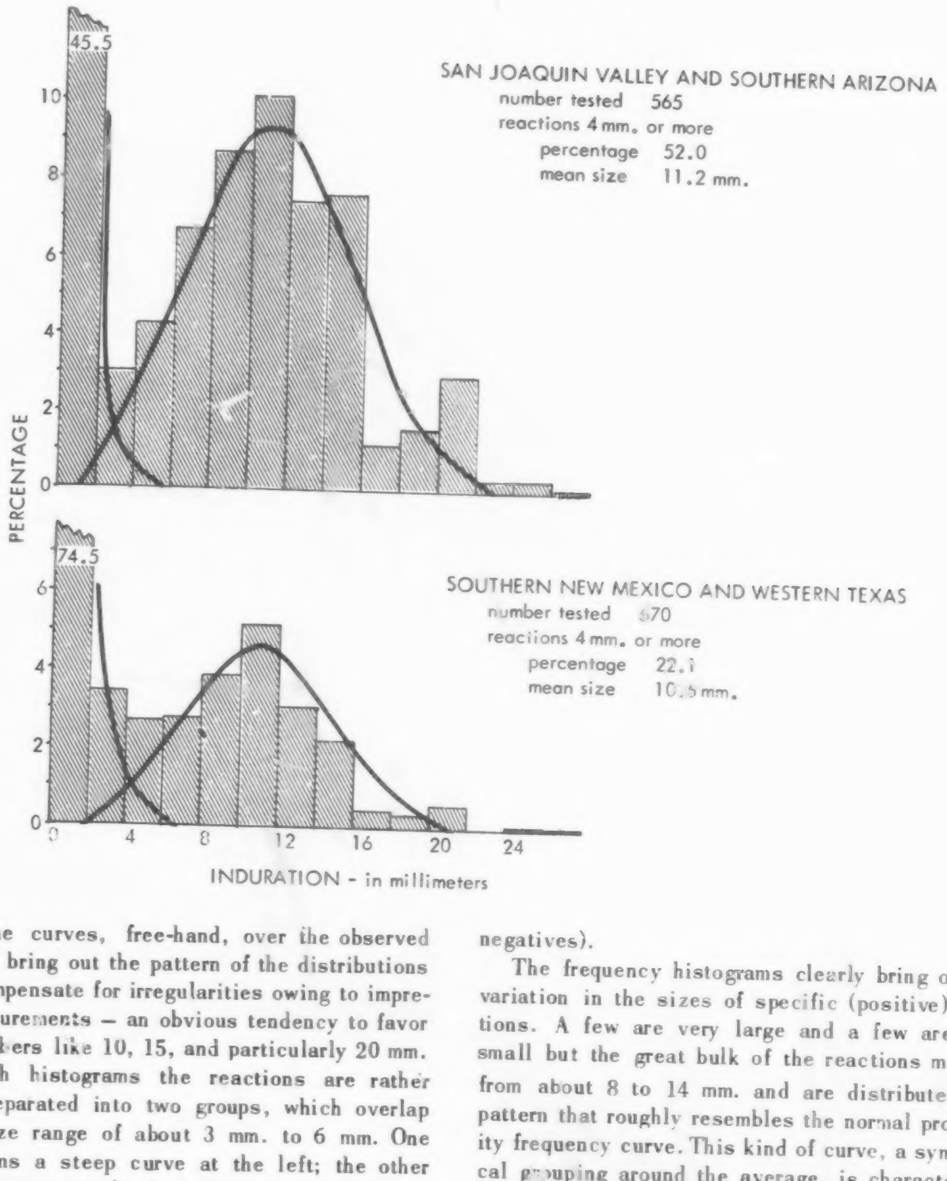

Percent Reactors
(5mm or more)
80-90
60-80
30-60
10-30
2-10
0- 2

Figure 2. Geographic distribution of histoplasmin sensitivity in young adults. (Reproduced with permission from *Diseases of the Chest*, Volume 29, 1956.)

FIGURE 3

COCCIDIOIDIN REACTIONS IN NAVY RECRUITS



smooth-line curves, free-hand, over the observed results to bring out the pattern of the distributions and to compensate for irregularities owing to imprecise measurements – an obvious tendency to favor round numbers like 10, 15, and particularly 20 mm.

In both histograms the reactions are rather clearly separated into two groups, which overlap in the size range of about 3 mm. to 6 mm. One group forms a steep curve at the left; the other forms a symmetrical, dome-shaped curve toward the right. We can assume that the reactions in the right-hand group represent specific sensitivity to coccidioidin – specific sensitization by infection with *C. immitis*. Those in the left-hand group with no induration at all, or an induration of only a few millimeters, represent the uninfected (the negatives).

The frequency histograms clearly bring out the variation in the sizes of specific (positive) reactions. A few are very large and a few are quite small but the great bulk of the reactions measure from about 8 to 14 mm. and are distributed in a pattern that roughly resembles the normal probability frequency curve. This kind of curve, a symmetrical grouping around the average, is characteristic of many kinds of biological data and it is not surprising that the sizes of skin reactions follow a pattern similar to other measurable biologic responses. Even though the proportion of positive reactions is 52 percent in one instance and only 22 percent in the other, the peak of each curve, which represents the average size of the positive reactions, differs by less than 1 mm. between the two distributions.

These results indicate that the average size of specific reactions to coccidioidin does not vary with the percentage of positive reactors. Whether 50 percent or only 20 percent of the population has been infected, the average reaction is close to 11 mm. in diameter. Repeated exposure to *C. immitis* infection (and we can probably assume that more persons will have been infected repeatedly in the higher than in the lower prevalence areas) apparently does not affect the size of the reactions. In other words, a large reaction to coccidioidin apparently does not denote more frequent infection, or a heavier infection, than a small reaction. Differences between the sizes of specific reactions of two persons must represent differences in biological response rather than differences in the frequency or intensity of exposure to coccidioidal infection.

We can also infer that sensitivity to coccidioidin does not wane or fade in the absence of repeated infection. If there were any significant waning of sensitivity, we would expect to find generally smaller reactions in localities where the infection rate is low, and the risk of repeated infection is therefore also low. Instead, we find no real difference in the average size of the reactions regardless of the prevalence of infection.

Frequency distributions of sizes of reactions to histoplasmin for lifetime residents of Missouri and Mississippi, two states in the *Histoplasma* endemic area, are shown in figure 4. In each distribution, the reactions are separated into two groups. The right-hand group forms a fairly symmetrical curve with a peak around 10 to 11 mm. These reactions can be regarded, we believe, as specific for histoplasmal infection. For Missouri the frequency of specific reactions is about 70 percent, for Mississippi about 40 percent; yet the mean size of specific reactions in each instance is just over 10 mm. Hence, as with specific coccidioidin sensitivity, the size of specific histoplasmin reactions does not appear to be influenced by the prevalence of infection.

Specific reactions thus appear to range in size from very large, more than 25 mm., to quite small, 5, 4, possibly 3 mm. But, for the other side of the picture, we ought to look for a moment at the sizes of negative reactions. Our material for lifetime residents of the Northwest, dealing with a population essentially negative to both antigens, is useful for that purpose.

Coccidioidin and histoplasmin tests were given to more than 5,000 recruits from five States in the Northwest (fig. 5). Most of the reactions are crowded over at the left-hand end of the scale. From the form of the distribution, we may infer that a high proportion of reactions measuring 5, 6, and even 7 mm. belong to the negative group. In contrast, when a high proportion of a population has positive reactions, as illustrated in figures 3 and 4, most of the 5, 6, and 7 mm. reactions belong to the positive group.

The few Northwesterners who had large reactions to coccidioidin or histoplasmin form a group that centers around 10 to 11 mm. We believe these large reactions are specific: they have the same average size as the specific reactions found in the endemic areas. As far as we know, neither coccidioidal nor histoplasmal disease has been diagnosed in a native resident of the Northwest. We may therefore presume that among the lifetime Northwesterners there are a few who may have taken short trips to the Southwest or to the east central area where they picked up an infection. Although the possibility of isolated, unrecognized foci of infection in the Northwest cannot be ruled out, it seems most likely that these few reactors (less than 1 percent of the total population) represent errors or deficiencies in our classification of lifetime residents.

On the problem of cross-reactions (nonspecific or heterologous reactions) figure 6 shows the sizes of coccidioidin reactions in the east central area where histoplasmal infection is highly prevalent and of histoplasmin reactions in the coccidioidal endemic area in the Southwest. As shown in the upper histogram, a few fairly large coccidioidin reactions are found in the east central area. These large reactions are probably specific for coccidioidal infection, and we no doubt find them in this region for the same reasons that we find them in the Northwest. In addition, the smaller reactions, those of 4 or 5 mm. up to 10 or even 12 mm., are a great deal more frequent than in the Northwest.

The correlation table between coccidioidin and histoplasmin reactions in the east central area shows that most of the small coccidioidin reactions are in people who also react to histoplasmin. Only a very few are found in people who do not react to histoplasmin. We can therefore interpret most of the small coccidioidin reactions as cross-reactions owing to histoplasmal infection.

The pattern for histoplasmin reactions in the Southwest is much like that for coccidioidin in the east central area, but there are two important

FIGURE 4

HISTOPLASMIN REACTIONS IN NAVY RECRUITS

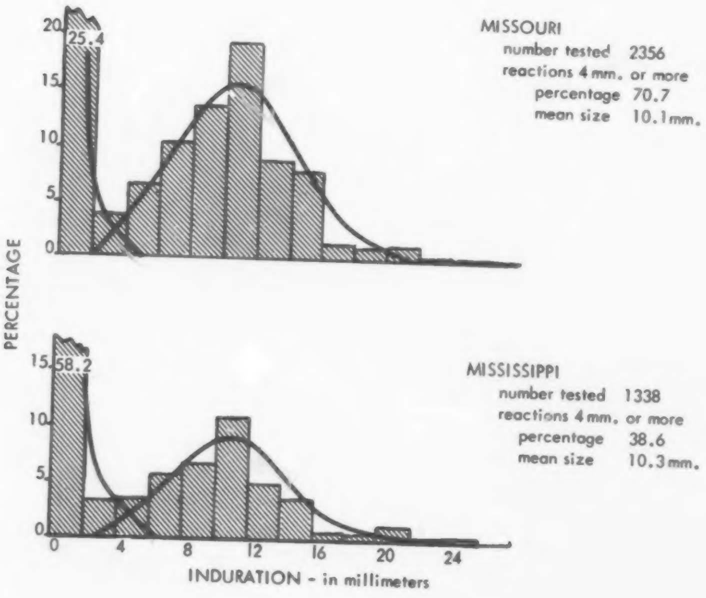


FIGURE 5

REACTIONS IN NAVY RECRUITS FROM FIVE NORTHWESTERN STATES

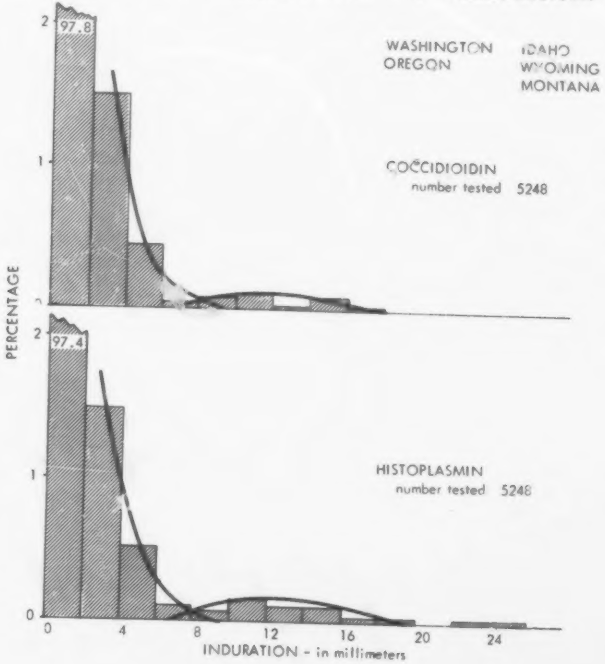
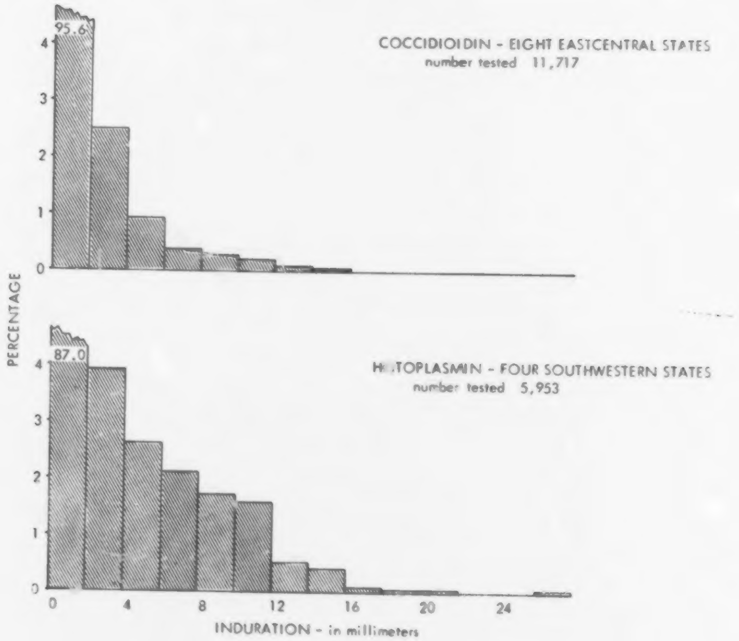


FIGURE 6

REACTIONS IN NAVY RECRUITS



differences:

First, there are proportionately more large histoplasmin reactions and the correlation table shows that a fair number of them are found in people who do not react to coccidioidin. We therefore cannot with any assurance exclude the possibility that some histoplasmin reactions in native residents of the Southwest are due to specific histoplasmal infection.

Second, there are a great many more small histoplasmin reactions in the Southwest than small coccidioidin reactions in the east central area. This finding is consistent with the expectation that histoplasmin cross-reactions are more frequent in coccidioidin reactors than coccidioidin cross-reactions are in histoplasmin reactors.

That most of these small histoplasmin reactions are very likely cross-reactions from coccidioidal infection is shown in figure 7, which gives results for lifetime residents of the San Joaquin Valley. The distribution of histoplasmin reactions, shown at the top for the total group, is very much like the previous one for the entire Southwest – there is a relatively large number of small reactions. In the lower section, the group is divided into two parts with those who do not react to coccidioidin on the left and those who do on the right. It is immediately evident that most of the small histoplasmin reactions are found in coccidioidin reactors and can therefore probably be interpreted as cross-reactions.

The distribution of histoplasmin reactions in coccidioidin nonreactors brings out another point. It was mentioned previously that at least some of the histoplasmin sensitivity in the Southwest is apparently independent of coccidioidin sensitivity. In the San Joaquin Valley, one of the regions of highest coccidioidin sensitivity, we find that about 5 percent of the persons who do not react to coccidioidin have sizable histoplasmin reactions. Some of these large reactions may well represent specific histoplasmin sensitivity acquired in the San Joaquin Valley.

In the course of analyzing the material from small geographic areas, we ran into a very unexpected finding for Navy recruits from an area in the eastern part of Texas within a radius of several hundred miles around Dallas and Fort Worth. Figure 8 gives a frequency distribution of histo-

FIGURE 7

HISTOPLASMIN REACTIONS IN NAVY RECRUITS FROM THE SAN JOAQUIN VALLEY, CALIFORNIA

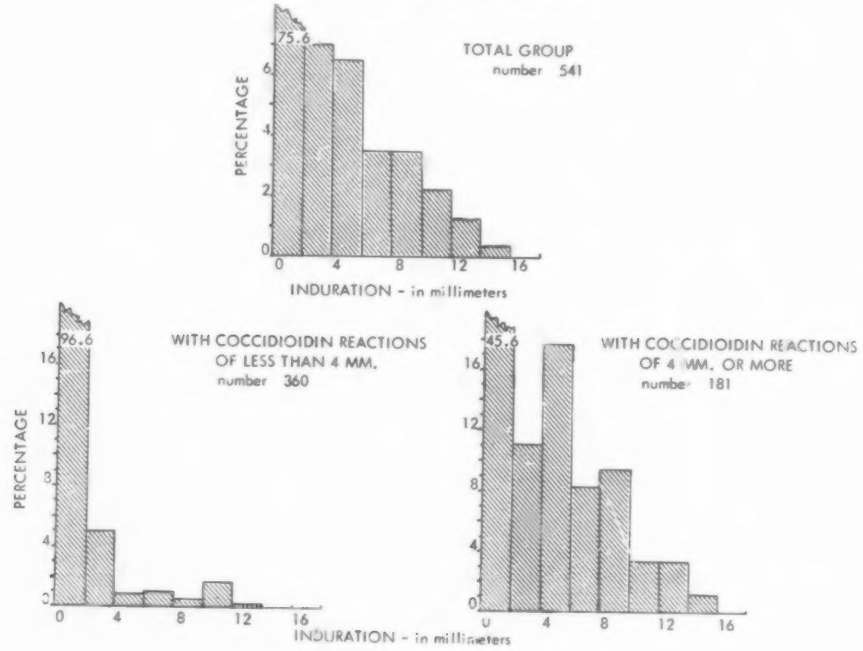
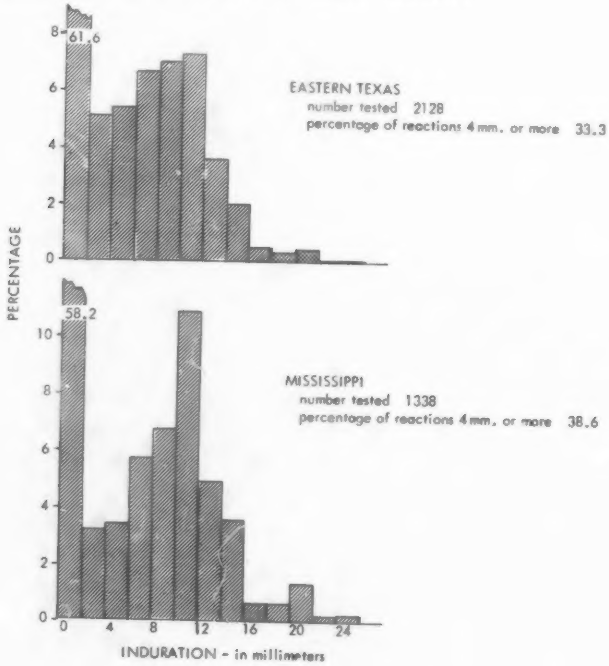


FIGURE 8

HISTOPLASMIN REACTIONS IN NAVY RECRUITS



plasmin reactions for more than 2,000 lifetime residents of that area. For comparison, the lower histogram is included to show histoplasmin reactions for lifetime residents of Mississippi. In both instances, about the same percentage of reactions measure 4 mm. or more, reactions that would ordinarily be called positive. Both areas are about the same distance from the heart of the endemic center for histoplasmosis. Yet, the patterns of the two distributions are quite different.

For Mississippi, as discussed previously, we have the dome-shaped curve characteristic of specific reactions, rising to a peak around 10 to 11 mm. and tapering off on both sides of the peak. For east Texas, we see toward the right the same tapering off from a peak at 10 to 11 mm.; but, instead of a symmetrical tapering off toward the left, the frequencies show very little decrease from 10 mm. down to 2 mm. In other words, we find a relative preponderance of small histoplasmin reactions in native residents of east Texas.

One might conclude that in east Texas we have an overlapping of the two endemic areas, that there is not only a moderately high prevalence of specific histoplasmin sensitivity but also histoplasmin cross-reactions due to coccidioidal infections. But this is an area where prevalence of coccidioidin reactors is very low, not more than 2 percent – a percentage entirely too low to account for the high frequency of small histoplasmin reactions.

As a matter of fact, the frequency of large coccidioidin reactions in east Texas and Mississippi is almost the same, as shown in figure 9. However, the relative proportion of small (nonspecific) coccidioidin reactions is higher in east Texas than in Mississippi. Moreover, since many of the small coccidioidin reactions in east Texas are found in histoplasmin nonreactors, they cannot be interpreted as cross-reactions.

We would postulate that these small coccidioidin reactions and the preponderance of small histoplasmin reactions represent the effect of some other infection, probably a fungus infection, existing in that region. Furthermore, it appears that the sensitivity caused by the unknown infection gives cross-reactions much more frequently with histoplasmin than with coccidioidin.

A schematic breakdown of the distribution of histoplasmin reactions in east Texas is shown in figure 10. (The upper histogram is the same as shown in figure 8.) Below, against a background of that same distribution, we have drawn free-hand curves to show the three different groups of reactions which we believe make up the distribution.

First, there is the group of negative reactions at the left, outlined by the broken-line curve. Then, there is the group of specific reactions toward the right, shown by the solid-line curve. Finally, as shown by the dotted-line curve, there is a group of nonspecific reactions interposed between the other two. The nonspecific reactions center around 7 mm. in size and overlap toward the right with the smaller specific reactions and toward the left with the larger negative reactions. These nonspecific reactions thus tend to fill in the gap otherwise found between the group of specific (positive) and the negative reactions.

We estimate that persons with nonspecific histoplasmin reactions make up nearly 15 percent of the total number of recruits who had lived all their lives in east Texas. But, because not all of the persons infected with the unknown fungus could be expected to show nonspecific (cross) reactions with histoplasmin, the prevalence of infection with the unknown organism may well be as high as 25 or even 30 percent. It would be our guess that if one tested with other fungus antigens in east Texas and the adjacent areas, it might be possible not only to find the cause but also to map out the geographic distribution of a presently unknown natural source of nonspecific histoplasmin sensitivity.

## Summary

The results of this study agree well with those of others – Smith's map(4) of several years ago, and also material presented at this Conference – that the *C. immitis* endemic area is probably limited to a well circumscribed area in the Southwest, ending fairly abruptly in the middle of Texas.

We can be less sure of the extent of the *H. capsulatum* endemic area, primarily because histoplasmin is a much less specific antigen than coccidioidin. Histoplasmin not only picks up specific sensitivity caused by histoplasmal infection; it also picks up a fair amount of the sensitivity caused by coccidioidal infection and, apparently, other infections as well. Although this is a disadvantage in trying to delineate the histoplasmal endemic area, it may have certain advantages in locating endemic foci of infection with other fungi.

There is little reason to doubt that *H. capsulatum* is endemic in the east central part of the country or that many other areas, such as the Northwest and the Southeast, are free of it. However, our

FIGURE 9

COCCIDIOIDIN REACTIONS IN NAVY RECRUITS

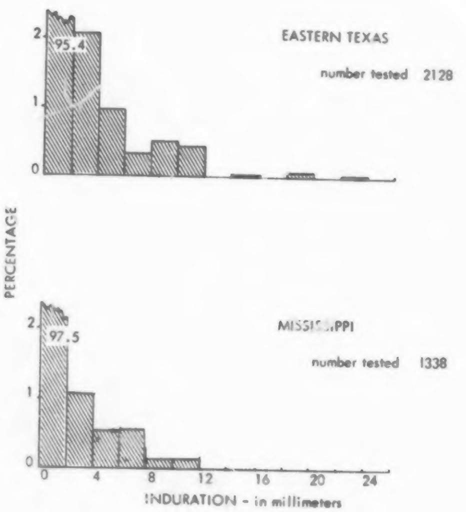
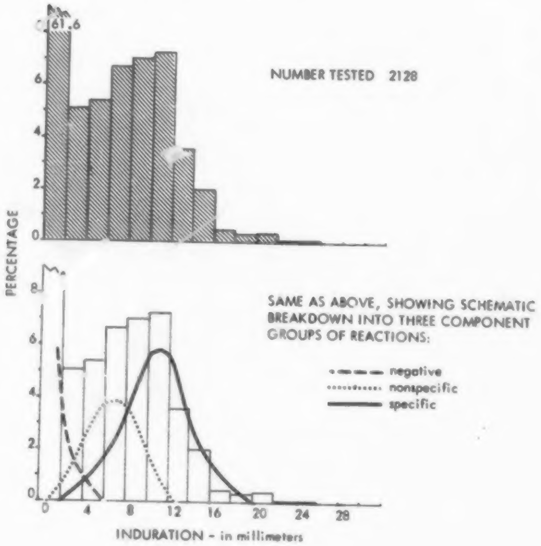


FIGURE 10

HISTOPLASMIN REACTIONS IN NAVY RECRUITS
FROM EASTERN TEXAS



results suggest that there may be sources of specific sensitivity to histoplasmin in the southwestern area, where there is not a high frequency but certainly a substantial one, running up to about 5 percent of large, apparently specific histoplasmin reactions in coccidioidin nonreactors.

Finally, our results lead directly to the hypothesis that some other sensitizing agent is responsible for some of the histoplasmin reactions in certain areas, as illustrated by the nonspecific histoplasmin sensitivity found in the Dallas-Fort Worth area of Texas. A carefully planned testing program with other fungus antigens in that area, together with a study of X-ray findings of people who have always lived there, could be expected to provide further information about other natural sources of histoplasmin sensitivity.

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## *Section V*

# Cultural And
# Cytologic Studies
# On Coccidioidomycosis

# The Production of Spherules in a Synthetic Medium by *Coccidioides immitis*

Bernard S. Henry, Ph.D., and Elizabeth M. O'Hern, Ph.D.

The development of a rapid means of identification of cultures of *Coccidioides immitis* and a rapid method of diagnosis of coccidioidomycosis is highly desirable.

Preliminary results of experiments now in progress show promise in the development of such a medium. Several factors necessary for stabilizing the test are, at present, unresolved. Among the factors limiting the use of the present synthetic medium is the unequal behavior of different strains of *C. immitis* in the medium.

## Materials and Methods

The strain of *C. immitis* used in all preliminary tests, No. 492, was isolated from a case of coccidioidomycosis in a human in 1952; it will be referred to as the control strain. The basic medium used initially in these studies is that of Baker *et al.*$^{(1)}$ with omission of copper sulfate. Changes made in the composition of this medium will be indicated in the section on results.

Scoring of the quantity of culture spherules was based roughly on the percentage of spherule clusters in relation to the total growth, a score of 2 being equivalent to 20 percent. This can be determined fairly accurately in young cultures, 3 to 5 days old, in which it is possible to examine the total growth. The method becomes less accurate in older cultures when aliquots of the total growth must be studied. Scores are given as the sum of scores of 5 tubes read at approximately 5 and 12 days.

All cultures were incubated at 37° C. An incubation atmosphere of 10 percent $CO_2$, 10 percent $O_2$, and 80 percent $N_2$ was obtained in culture jars or in a sealed incubator. Each test consisted of 5 tubes containing 5 ml. of medium.

Spherule suspensions used for inocula were prepared by mincing infected mouse brain in saline, followed by washing three times with saline. Arthrospore suspensions were collected in a syringe by washing the surface of a culture grown on a Sabouraud's agar slant in a tube capped with a rubber sleeve-type stopper. About 0.1 ml. of such suspensions was used as inoculum.

Additional strains of *C. immitis* were obtained through the courtesy of Dr. Libero Ajello of the Communicable Disease Center in Chamblee, Georgia, and through the courtesy of Dr. C. E. Smith, Dean, School of Public Health, University of California, Berkeley, California. Numbers and names used to differentiate these strains are those used by the respective sources.

## Results

Tests have shown glucose to be preferable to the acetate used in the initial medium for spherule production (fig. 1). A concentration of 1 to 4 percent appears to be satisfactory. Ammonium chloride in 0.1 to 1.0 percent is favorable to spherule production; many of the spherules in media containing less than 0.1 percent ammonium chloride are vacuolated. An acid pH is necessary for spherule production in this medium, with a range from pH 5.5 to pH 6.5 giving best results. The relative concentration of the phosphate buffer salts was changed from that of the initial medium to bring the pH of the medium to approximately 6.0 without further adjustment, although pH adjustment to 6.0 was made with NaOH or HCl when necessary. The resulting basal medium, No. 14, has the following composition: glucose, 2 percent; ammonium chloride, 1 percent; primary potassium phosphate, 0.6 percent; secondary sodium phosphate, 0.3 percent; magnesium sulfate, 0.01 percent.

Inocula consisting of freshly prepared spherule suspensions yielded the greatest quantity of culture spherules in the basal medium. Older suspensions became increasingly less satisfactory. Tissue spherule inocula more than 1 month old yielded no spherules in culture (table 1), although

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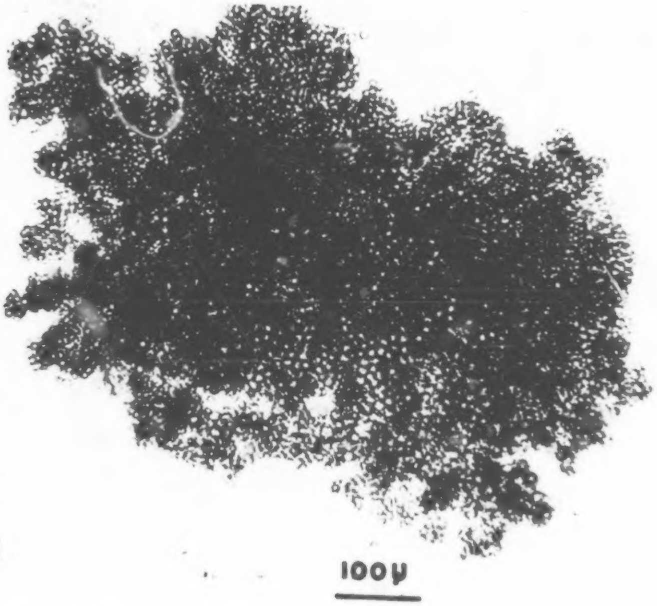

Figure 1. Spherule produced by use of glucose in the initial medium.

Table 1

EFFECT OF AGE OF INOCULUM ON *IN VITRO* SPHERULE PRODUCTION

| Inoculum | Spherule Score | |
|---|---|---|
| | Basal medium | Basal medium plus 0.1 ml. FMB* per tube |
| 2-day MBS* | 13 | 2 |
| 38-day MBS | 0 | 0 |
| 130-day MBS | 0 | 0 |
| Hyphae | 16 | 7 |

*Fresh mouse brain suspension in saline.

**Mouse brain spherules.

spherules were produced in serial subcultures (table 2). Culture spherules were produced somewhat irregularly and generally in smaller quantities when arthrospores were used as inocula. In serial subcultures the quantity of spherules increased in most instances (table 2). Cultures in basal medium did not produce spherules with either spherule or arthrospore inoculum when incubated in air.

Addition of purines and pyrimidines to the basal medium improved the output of culture spherules from arthrospore inocula relative to spherule inocula, so that the unknown tissue factor could be omitted from the cultures. Of the compounds tested (table 3), cytosine gave the best results quantitatively and qualitatively, for spherules produced in this medium showed secondary development – a second generation of spherules (fig. 2). With the cytosine medium a candle jar was found to give almost as satisfactory an environment as an atmosphere of 10 percent $CO_2$, 10 percent $O_2$ in $N_2$.

Tests of 17 strains of *C. immitis* in cytosine

TABLE 2

*IN VITRO* SPHERULE PRODUCTION IN SERIAL SUBCULTURE

| Inoculum | Scores of subcultures at 8-12 day intervals |
|---|---|
| 77-day MBS* | 0-4-2 |
| Subculture at 6 days of 3-day MBS with score of 29 | 18-5-7 |
| Hyphae | 6-10-11 |

* Mouse brain spherules.

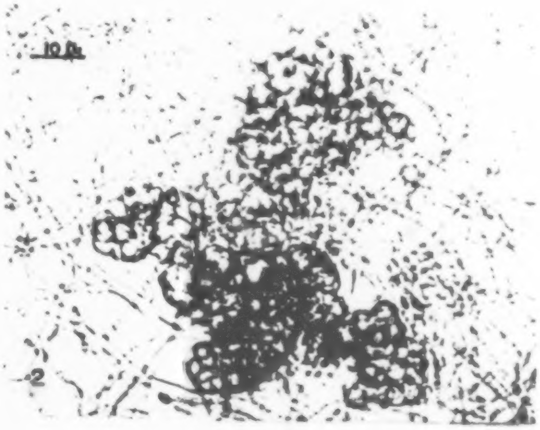

Figure 2. Second generation of spherules produced in cytosine medium.

TABLE 3

EFFECT OF PURINES AND PYRIMIDINES ON PRODUCTION OF CULTURE SPHERULES

| Test Compound | Concn. µg/ml. | Scores | | | |
|---|---|---|---|---|---|
| | | 10% $CO_2$ Hyphae | 10% $O_2$ MBS* | Incubated in Air Hyphae | Incubated in Air MBS |
| Hypoxanthine | 1 | 4 | 1 | 0 | 0 |
| Xanthine | 1 | 10 | 4 | 0 | 0 |
| Adenine | 1 | 10 | 0 | 0 | 0 |
| Guanine | 1 | 10 | 2 | 0 | 0 |
| Uracil | 0.5 | 6 | 2 | 0 | 0 |
| Thymine | 5 | 15 | 15 | 2 | 3 |
| Cytosine | 5 | 29 | 22 | 1 | 2 |
| None | - | 8 | 10 | 0 | 0 |

* Mouse brain spherules.

medium showed a considerable difference in ability to produce culture spherules (table 4). Tissue spherule inocula produced culture spherules in 9 strains; of these, 5 produced culture spherules when arthrospores were used as inocula. Antibiotics – 100 units each of penicillin, streptomycin, and cycloheximide – were used in tests as indicated in the table. They appear to have suppressed development of culture spherules in controls (table 5) and thus make such tests not equivalent to those without antibiotics. The age of the cytosine stock solution was found to affect results in control tests (table 5); thus, this factor must also be weighed in the results with the several strains.

## TABLE 4
## SPHERULE PRODUCTION BY STRAINS OF *C. IMMITIS* IN CYTOSINE MEDIUM

| Strain | Inoculum | Age Basal Medium Days | Age Stock Cytosine Days | Score | Date of Test |
|---|---|---|---|---|---|
| A-384 | MBS* | 10 | 5 | 24 | 19 Nov |
| A-384 | Hy** | 10 | 27 | 24 | 11 Dec |
| A-453 | MBS | 10 | 5 | 11 | 19 Nov |
| A-453 | Hy | 10 | 27 | 0 | 11 Dec |
| A-659 | MBS | 1 | 2 | 0 | 16 Jan |
| A-659 | Hy | 1 | 2 | 0 | 16 Jan |
| A-429 | MBS | 17 | 12 | 7½ | 26 Nov |
| A-429 | Hy | 32 | 27 | 0 | 11 Dec |
| 901-56 | MBS | 7 | 37 | 1 | 21 Dec*** |
| 901-56 | Hy | 7 | 54 | 0 | 7 Jan |
| B-13 | MBS | 17 | 12 | 12 | 26 Nov |
| B-13 | Hy | 32 | 27 | 1 | 11 Dec |
| Silveira | MBS | 8 | 23 | 0 | 17 Dec*** |
| Silveira | Hy | 18 | 47 | 0 | 31 Dec |
| Bates | MBS | 38 | 33 | 3 | 17 Dec*** |
| Bates | Hy | 18 | 47 | ½ | 31 Dec |
| Cash | MBS | 38 | 33 | 0 | 17 Dec*** |
| Cash | Hy | 18 | 47 | 0 | 31 Dec |
| Renner | MBS | 38 | 33 | 12 | 17 Dec*** |
| Renner | Hy | 18 | 47 | 4 | 31 Dec |
| Lloyd | MBS | 38 | 33 | 0 | 17 Dec*** |
| Lloyd | Hy | 18 | 47 | 0 | 31 Dec |
| King | MBS | 18 | 37 | 0 | 21 Dec*** |
| King | Hy | 24 | 54 | 0 | 7 Jan |
| Soil 3a | MBS | 18 | 37 | 1 | 21 Dec*** |
| Soil 3a | Hy | 24 | 54 | 0 | 7 Jan |
| Marter | MBS | 18 | 37 | 2 | 21 Dec*** |
| Marter | Hy | 24 | 54 | ½ | 7 Jan |
| Thomas | MBS | 18 | 37 | 0 | 21 Dec*** |
| Thomas | Hy | 1 | 2 | 0 | 16 Jan |
| Norman | MBS | 18 | 37 | 0 | 21 Dec*** |
| Norman | Hy | 1 | 2 | 0 | 16 Jan |
| Sanders | MBS | 18 | 37 | 0 | 21 Dec*** |
| Sanders | Hy | 24 | 54 | 0 | 7 Jan |

*Mouse brain spherules.

**Hyphae.

***Antibiotics.

## TABLE 5
## CONTROL CULTURES IN CYTOSINE MEDIUM
Arthrospore Inoculum

| Date of Test | Age Basal Medium Days | Age Stock Cytosine Days | Score | |
|---|---|---|---|---|
| | | | Candle Jar | Air |
| 19 Nov | 10 | 5 | 27 | |
| 19 Nov | 10 | 5 | | 3 |
| 26 Nov | 17 | 12 | 2½ | |
| 26 Nov | 17 | 12 | | 0 |
| 11 Dec | 32 | 27 | 13 | |
| 11 Dec | 32 | 27 | | 3 |
| 17 Dec* | 38 | 33 | 4 | |
| 17 Dec* | 38 | 33 | | 2 |
| 31 Dec | 18 | 37 | 14 | |
| 31 Dec | 18 | 37 | | 0 |
| 7 Jan | 25 | 54 | 6 | |
| 7 Jan | 25 | 54 | | 0 |
| 15 Jan | 1 | 1 | 18 | |
| 15 Jan | 1 | 1 | | 1 |
| 15 Jan | 1 | 62 | 5 | |
| 15 Jan | 1 | 62 | | 0 |
| 15 Jan | 1 | - | 8 | |
| 15 Jan | 1 | - | | 0 |
| 21 Dec* | 7 | 37 | 0 | |
| 21 Dec* | 7 | 37 | | 5½ |
| 21 Dec | 7 | 37 | 14 | |

* Antibiotics.

### Discussion

It has been shown that increased $CO_2$ is a requirement for spherule production by *C. immitis* in other successful tests.(2) Purines and pyrimidines have been shown to substitute for such a requirement in streptococci,(3) and in *Neisseria gonorrhoeae* after primary isolation.(4) Although labelled $CO_2$ was found to be fixed in nucleic acids by *Brucella abortus*, purines and pyrimidines could not substitute for the $CO_2$ requirement.(5) The purines and pyrimidines tested with *C. immitis* appear not to have replaced the requirement for increased $CO_2$ in this organism but to have enhanced the $CO_2$ effect on production of culture spherules.

Strain differences in *C. immitis* in respect to virulence(6) and sporulation capacity(7) have recently been reported. Variation among strains in their capacity to produce spherules in cultures adds to the unstable factors which should be considered in studying this organism.

### Summary

A synthetic medium for the production of culture spherules by *C. immitis* under specified cultural conditions is described. Addition of cytosine to this medium enhances spherule production. Of 17 strains of the organism tested in the cytosine medium, 9 produced culture spherules when tissue spherules were used as inoculum and 5 produced culture spherules when arthrospores were used as inoculum.

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## Acknowledgements

We wish to acknowledge the excellent technical assistance of Mrs. Anne Ulbright.

# Histochemical Studies on *Coccidioides immitis*

A. M. Breslau, M.D.

For several years studies have been carried out on the histochemistry of *Coccidioides immitis*. The purpose of this brief report is to point out, with a few photographs, how such investigations can cast light on the development of the parasite and its relation to host responses.

Mature sporangia of *C. immitis* are surrounded by a thick phospholipid layer (fig. 1). This substance is revealed when tissue sections are stained by Baker's modification of the Smith-Dietrich reaction. Slides fixed with Bouin's fluid and treated with pyridine prevent staining of this layer. These results are corroborated by Doctors O'Hern and Henry's electron microscope studies.[1] Whether this phospholipid is produced by the parasite or by the host remains undetermined. But it must be noted that this layer cannot be demonstrated in the endospores or the young spherules; it develops with the maturation of the spherule. The wall of the spherule (fig. 2) reacts vigorously with the Bauer's or McManus' technique. The reaction is blocked by acetylation and does not occur if chromic acid or periodic acid oxidation is omitted, proving that the substance is a polysaccharide and not a fatty aldehyde. The wall is not affected by treatment with Schweitzer's reagent, a cellulose solvent (fig. 3), but it is degraded by chlorine which attacks chitin. The wall, therefore, contains chitin, but careful study shows it to contain other polysaccharides as well as lamellae of protein. It is these other polysaccharides which must be the main antigenic agents, and it may be that the presence of the phospholipid layer prevents their stimulating the production of antibodies.

Figure 4 shows a *C. immitis* sporangium stained with toluidine blue buffered to pH 4.5. The wall of the sporangium is green. The nature of the cause of this type of metachromasia is unknown to me. Interesting also is the metachromasia within the walls of the parasite. This reaction is found in all stages of sporangial development. In the small spherules, the substance causing this reaction seems capable of migrating through the wall into the surrounding tissue. Tarbet and Breslau[2] showed that when young spherules only were present, the cellular response of the host was composed largely of polymorphonuclear neutrophils.

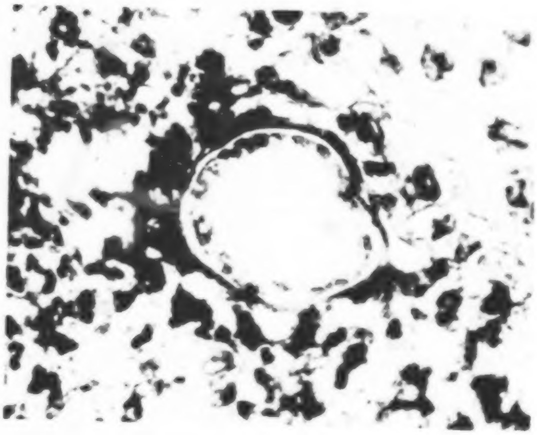

**Figure 1.** Sporangium with colorless wall surrounded by a shell of phospholipid.

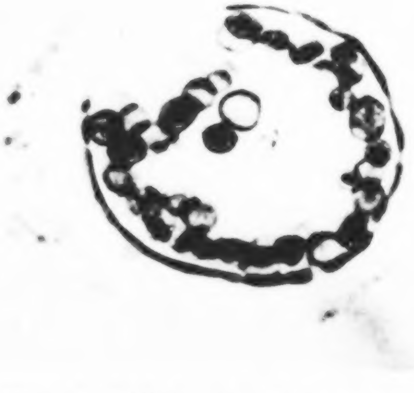

**Figure 2.** Gridley's stain. Walls of spherule and endospores stained. Polysaccharide also present within endospores.

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*From the Veterans Administration Center and University of California Medical Center, Los Angeles, California.*

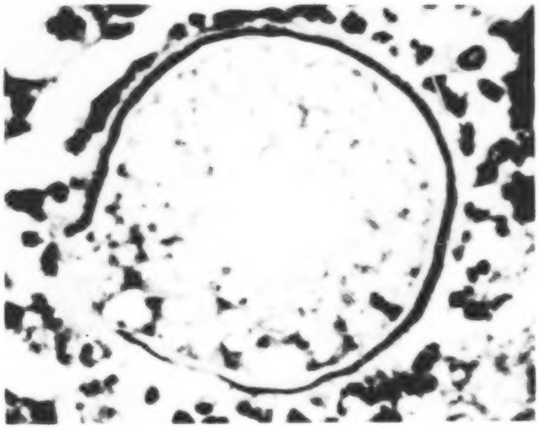

Figure 3. Schweitzer's reagent followed by safranine and fast green. Cell wall not dissolved. Stained by safranine and surrounded by basophilic layer.

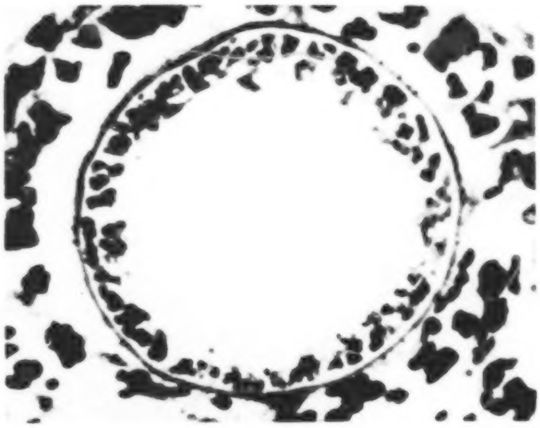

Figure 4. Korson's trichome stain to show ribonucleic acid. Ribonucleic acid within cytoplasm of spherule.

When older spherules with external phospholipid are present, the metachromatic substance is retained within the spherule, and the cellular response is changed to monocytic. When the development is no longer synchronous, and both large and small spherules are present, the response is mixed. Ribonuclease does not remove – if anything it enhances – the metachromatic reaction, proving the presence of acid mucopolysaccharide. Hyaluronidase does not extinguish this reaction.

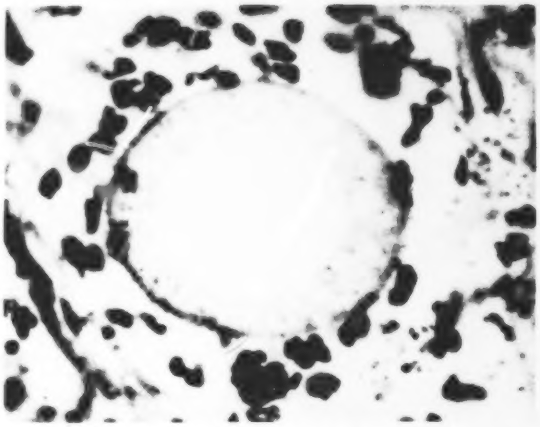

Figure 5. Ribonuclease followed toluidine blue. Ribonucleic acid removed from cytoplasm unmasking nuclei and metachromasia (acid mucopolysaccharide).

The phospholipid layer may, therefore, control the nature of the response by the host by retaining the acid mucopolysaccharide within the spherule and by separating the wall from the host.

Finally, in a section stained with Korson's trichrome, we see large masses of stainable substance blocking the view of underlying structures (fig. 5). Ribonuclease removes this substance, which must therefore be ribonucleic acid (R.N.A.), and unmasks the minute nuclei. The presence of R.N.A. indicates the active protein synthesis throughout the life cycle of the parasite.

With the extension of analytical techniques in electron microscopy, and with the introduction of more specific histochemical techniques, we should be able to get a more satisfactory picture of the metabolism of the parasitic phase of *C. immitis.*

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# An Electron Microscope Study of *Coccidioides immitis*

Elizabeth M. O'Hern, Ph.D., and Bernard S. Henry, Ph.D.

Electron microscopy of thin sections of tissues and organisms has opened a vast field for study. It has made possible a mapping of cell structure and thereby enriched the vocabulary of cytology. It has aided in the understanding of interrelationships among cells and has, in some instances, suggested the function of cell organelles. Further and comparative studies of cellular fine structure should contribute to establishing taxonomic relationships among organisms.

The study of thin sections of *Coccidioides immitis* has been illuminating in revealing the basic structure of cell components. It has suggested the mode of cross-wall formation and the steps in cleavage and endospore formation, but it has merely introduced the study of many features found in the interesting developmental cycle of this organism. Advances in techniques of specimen preparation for electron microscopy continue to enhance the condition of the material under study and to improve the structural detail in electron micrographs.

## Methods

Spherules were freed from tissue by trypsin digestion prior to fixation.(1) This procedure was used to insure finding sufficient numbers of spherules for study in the embedding plastic. At present we are staining approximately 1.0μ thick methacrylate sections of other material with a 1-3 dilution of Giemsa stain, (which is made by combining 1 part Giemsa stock, ½ part methyl alcohol, 1½ parts phosphate buffer at pH 6.8, letting it stand for 10 minutes, then filtering it). With this method it is possible to become oriented in tissue and to trim blocks to the desired area for thin sections. Thus, spherules might be fixed *in situ* and studied in relation to the tissue.

The rupture of the cell walls of bacteria and fungi during the polymerization process has been persistently troublesome in electron microscope studies of these organisms. Two new embedding media have recently been proposed(2, 3) which may overcome this particular obstacle to good electron micrographs of plant tissue.(4)

It is therefore reasonable to anticipate improvements in electron microscopy that will make a great contribution to the knowledge of the structure of microorganisms and their relationships as well.

## Results and Discussion

Both hyphal and spherule nuclei of *C. immitis* appear to be similar (figs. 1, 2). Ranging from 0.5μ to 2.0μ in diameter, the nuclei are limited by a double membrane sometimes seen to contain pores. Similar nuclei may be seen in the fungus *Allomyces*.(5) Nucleoli are frequently present (figs. 2, 3). They vary in particle size and density and are usually in contact with the nuclear membrane. In multinucleate spherules the nuclei tend to be distributed near the cell periphery, while in younger spherules nuclei occupy a central position.

Mitochondria observed in the filamentous phase of *C. immitis* tend to be elongate, sometimes lined up alongside a narrow nucleus (fig. 4). In spherules the mitochondria are most usually found to be smaller and more nearly spherical. Many of the mitochondria are limited by a complete outer membrane and have cristae arising from an inner membrane (fig. 5), as is usual in other cells and organisms.(6) Occasionally mitochondria are found with an outer membrane recurved onto the cristae making them continuous with the cytoplasm. An example of this type may be seen in figures 2 and 6 where mitochondrion are adjacent to apparently well-fixed nuclei. It may be significant that this type of mitochondrion is found in a multinucleate spherule prior to the appearance of cleavage furrows, for Dempsey(7) has described open mitochondria in the cells of differentiating tissue. The nebenkern, a body believed to be formed from

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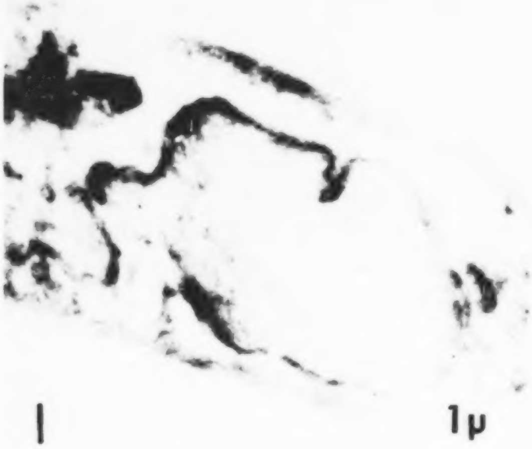
*From the Department of Microbiology, University of Washington School of Medicine, Seattle, Washington.*



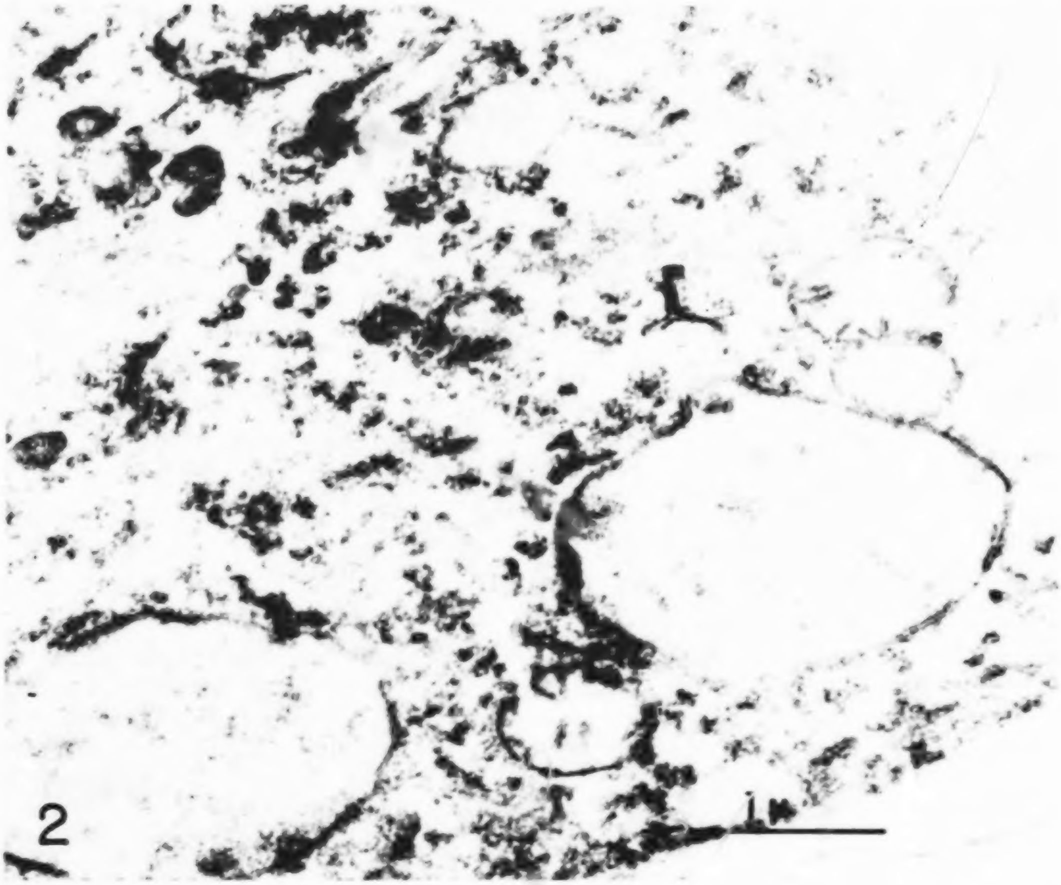


Figure 1. Hypha from 4-day culture: nucleus. x 38,000.

Figure 2. Tissue spherule: nuclei, nucleoli, and mitochondria. x 36,000.

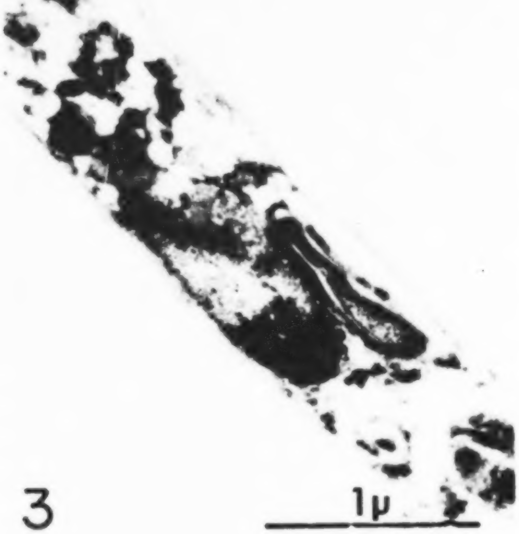


Figure 3. Hypha from 4-day culture: lobulate nucleus with nucleolus. x 32,000.

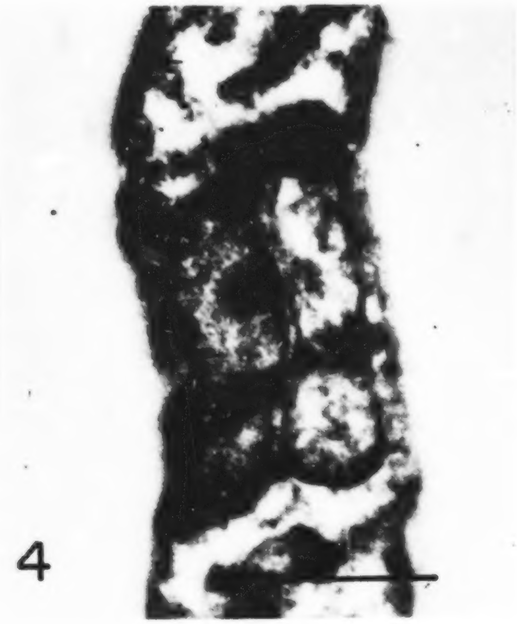


Figure 4. Hypha from 4-day culture: contiguous nucleus and mitochondrion. x 32,000.

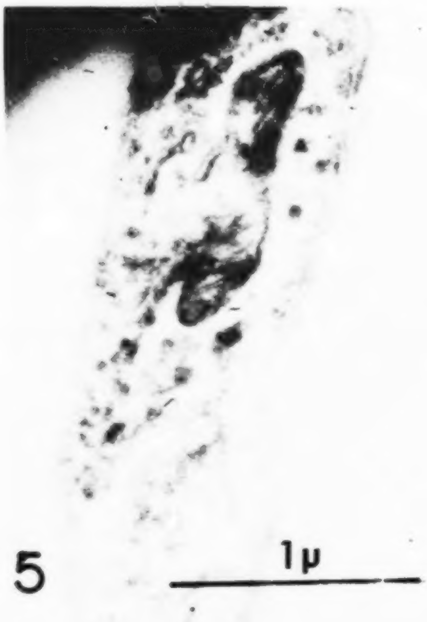


Figure 5. Hypha from 4-day culture: mitochondria and convoluted cytoplasmic membrane. x 38,000.

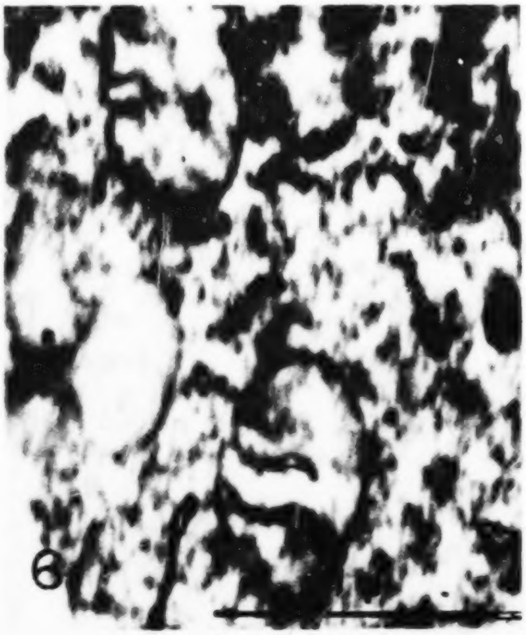


Figure 6. Tissue spherule: mitochondria with infolded membranes. x 45,000.

mitochondria, appears to have areas open to the cytoplasm.(8) This body, too, is found during a differentiation process such as the formation of fibrils of the spermatozoan tail of the grasshopper.

Cross-wall formation in hyphae appears to be initiated by the cytoplasmic membrane (fig. 7) which subsequently is found to be double (fig. 8) with wall material deposited between the membranes. This inner, less opaque layer of the septum increases in thickness, and in arthrospores becomes divided by another dense membrane, probably an abscission layer (fig. 9). This method of cross-wall formation is classically referred to as division by partition.(9) We have elsewhere suggested(1) that a single membrane may cut through the cytoplasm, then split and deposit the wall material between membranes. However, the deposition of several layers of material between adherent cell membranes has been described(10) as occurring during the development of a hair follicle. A similar process may occur in the formation of the hyphal septum: the adhesion of infolded cytoplasmic membranes may precede the deposition of wall material.

During the early stages of cross-wall formation dense septal granules are found near or overlying the cross-wall. Granules of similar appearance have been found in association with the walls of the alga *Phormidium*.(11) These granules are not found in association with older, well-developed septa of *C. immitis*.

A convoluted cytoplasmic membrane can be observed in some cells (fig. 10). Older cells of the hyphae seem to lack this feature, thus it may be associated with a metabolic process, such as wall formation, or with volume changes within the cell. Convolutions of the cytoplasmic membrane were not observed in spherules, but the spherule wall was frequently ruptured.

The cytoplasmic membrane appears also to be concerned with the formation of cleavage furrows (fig. 11). It precedes the wall material in segmenting the cytoplasm. The furrows are thicker near the wall (fig. 12). It appears that here too the wall material is deposited between two membranes. The furrows often branch near the cell periphery before the center of the spherule has been segmented. No granules equivalent to septal granules have been found in cleaving spherules; mitochondria are, however, present in large numbers.

The walls of spherules contain an outer, dense, capsular layer. On small cells this layer is irregular, but on larger spherules the layer becomes thicker and organized into club-like projections (fig.

7

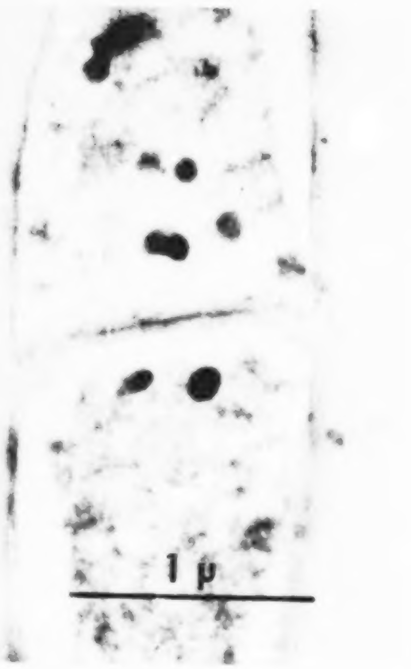


Figure 7. Hypha from 4-day culture: early septum formation. x 36,000.

8

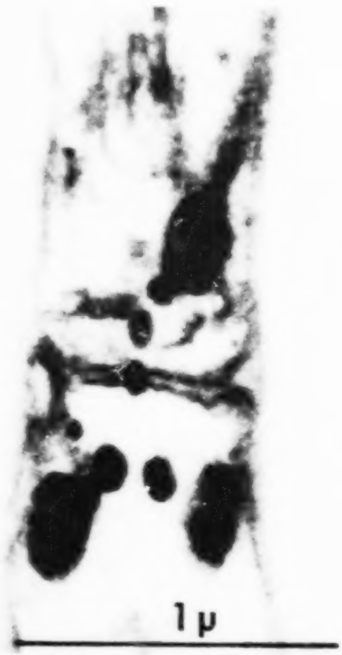


Figure 8. Hypha from 4-day culture: later development of septum; two types of septal granules. x 48,000.

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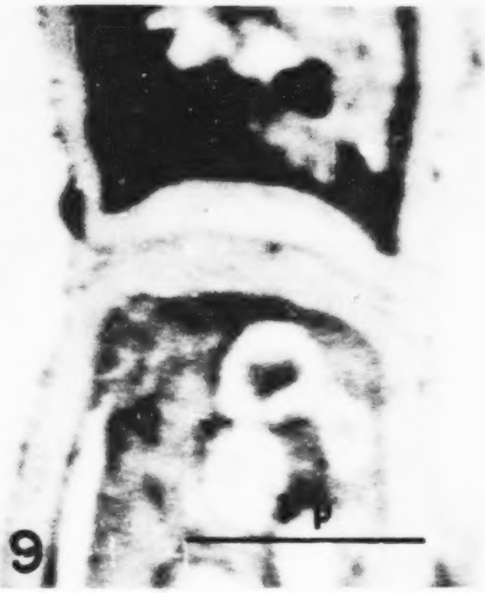

Figure 9. Hypha of 20-day culture: advanced development of septum; lipid granules. x 36,000.

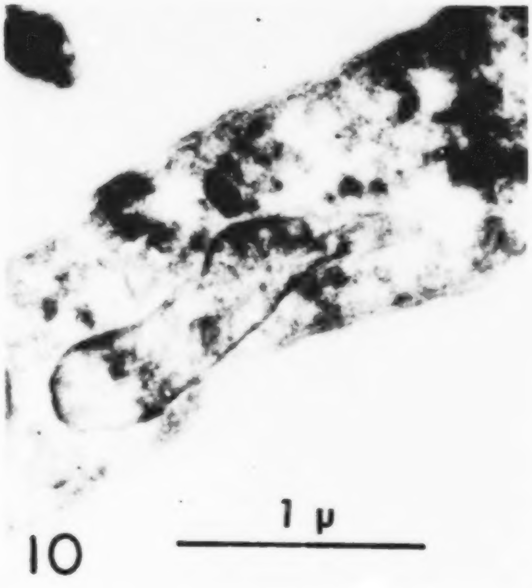

Figure 10. Hypha from 4-day culture: mitochondria and convoluted cytoplasmic membrane. x 38,000.

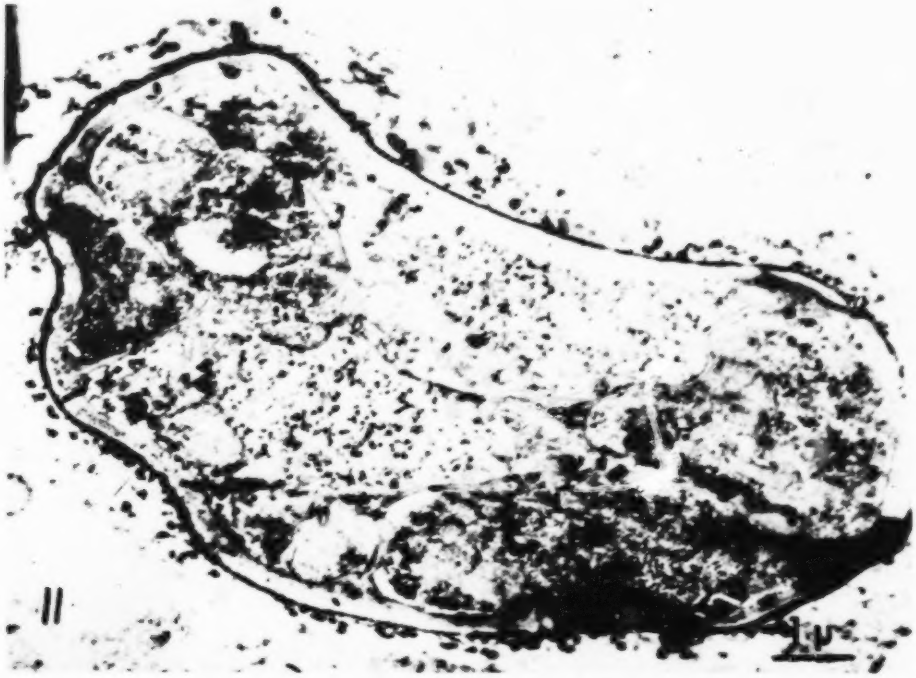

Figure 11. Tissue spherule in cleavage showing capsular layer. x 10,000.

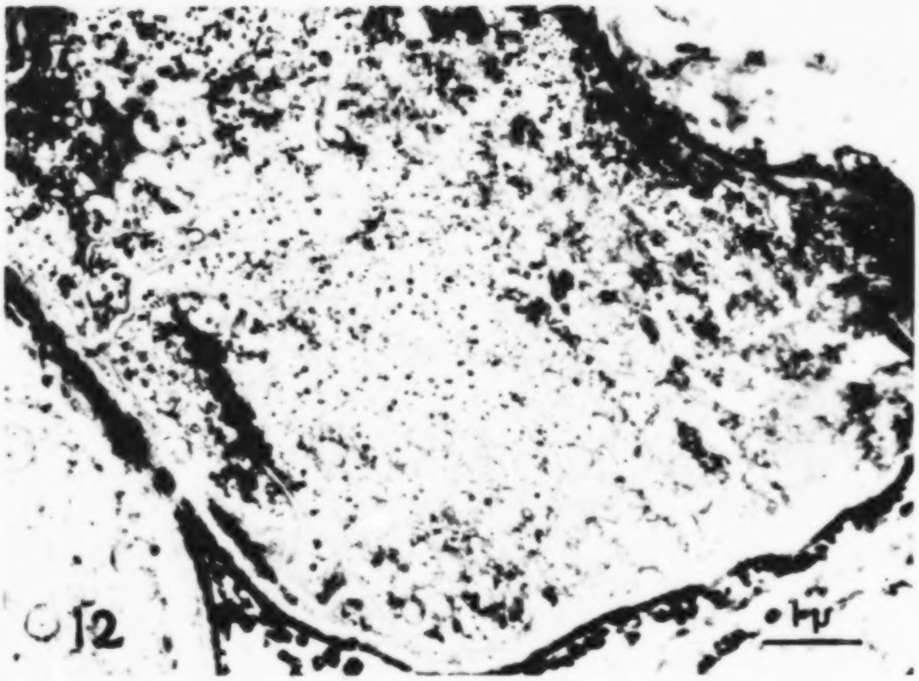

Figure 12. Tissue spherule in cleavage: branching cleavage furrows; mitochondria. x 12,000.

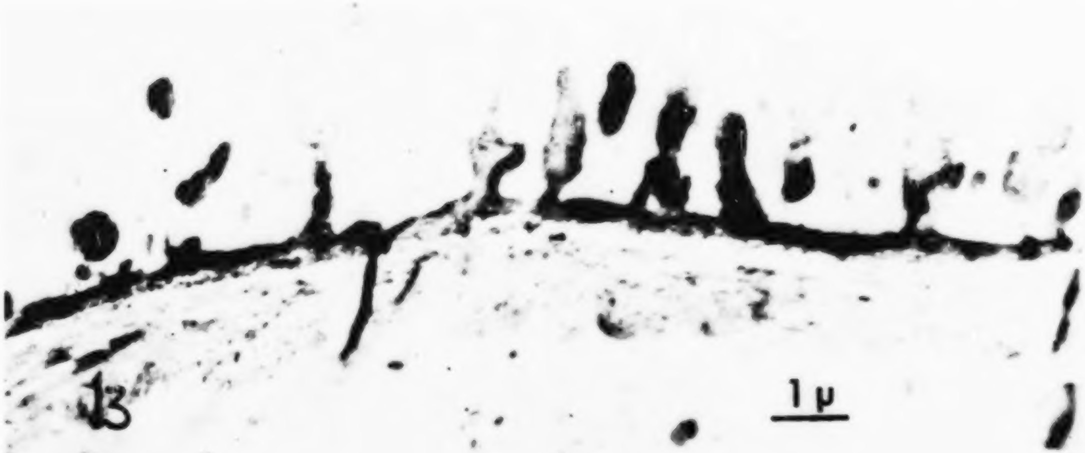

Figure 13. Large tissue spherule: capsule layer and wall clubs. x 12,000

13). Unlike the wall material which is fibrillar, the outer, capsule layer appears finely granular.

The walls of *C. immitis* have been described as being chitinous⁽¹²⁾ and the capsular layer may be the phospholipid demonstrated histochemically by Tarbet and Breslau.⁽¹³⁾ This layer also stains with lipid stains under certain conditions.⁽¹⁴⁾

In summary, electron micrographs of sections of *C. immitis* have revealed nuclei surrounded by a double membrane with pores, typical mitochondria, and occasional open mitochondria; and the details of the process of formation of cross-walls and cleavage furrows have been suggested.

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